FORM-PTO-1390 (Rev. 12-29-99) DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

# TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER
001560-397

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)
To be assigned 09/830123

| INTERNATIONAL APPLICATION NO. | INTERNATIONAL FILING DATE | PRIORITY DATE CLAIMED |
|---|---|---|
| PCT/JP00/05722 | 24 August 2000 | 24 August 1999 |

TITLE OF INVENTION
GENES ENCODING PROTEINS REGULATING THE pH OF VACUOLES

APPLICANT(S) FOR DO/EO/US
Shigeru IIDA, Sachiko TANAKA, and Yoshishige INAGAKI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☒ has been transmitted by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☐ have been transmitted by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11. to 16. below concern other document(s) or information included:**

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    International Search Report
    Sequence Listing (paper copy)
    Japanese PCT Request Form
    PCT Notice Informing the Applicant of the Communication of the International Application to the Designated Offices (Form PCT/IB/308)
    Cover page from published PCT international application (WO 01/14560)

| U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.50) | INTERNATIONAL APPLICATION NO. | ATTORNEY'S DOCKET NUMBER |
|---|---|---|
| To be assigned 09/830123 | PCT/JP00/05722 | 001560-397 |

| | CALCULATIONS | PTO USE ONLY |
|---|---|---|
| 17. ☐ The following fees are submitted: | | |
| **Basic National Fee (37 CFR 1.492(a)(1)-(5)):**<br>Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .......... $1,000.00 (960)<br>International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .......... $860.00 (970)<br>International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .......... $710.00 (958)<br>International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) .......... $690.00 (956)<br>International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) .......... $100.00 (962) | | |
| **ENTER APPROPRIATE BASIC FEE AMOUNT =** | $ 860.00 | |
| Surcharge of **$130.00 (154)** for furnishing the oath or declaration later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(e)). | $ | |

| Claims | Number Filed | Number Extra | Rate | | |
|---|---|---|---|---|---|
| Total Claims | 51 -20 = | 31 | X$18.00 (966) | $ 558.00 | |
| Independent Claims | 3 -3 = | 0 | X$80.00 (964) | $ | |
| Multiple dependent claim(s) (if applicable) | | | +$270.00 (968) | $ | |

| | | |
|---|---|---|
| **TOTAL OF ABOVE CALCULATIONS =** | $ | |
| Reduction for 1/2 for filing by small entity, if applicable (see below). | $ | - |
| **SUBTOTAL =** | $ 1,418.00 | |
| Processing fee of **$130.00 (156)** for furnishing the English translation later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(f)). + | $ | |
| **TOTAL NATIONAL FEE =** | $ 1,418.00 | |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 (581)** per property + | $ 40.00 | |
| **TOTAL FEES ENCLOSED =** | $ 1,458.00 | |
| | **Amount to be: refunded** | $ |
| | **charged** | $ |

a. ☐ Small entity status is hereby claimed.

b. ☒ A check in the amount of $ 1,458.00 to cover the above fees is enclosed.

c. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $______ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

for Donna M. Meuth
NAME

36,607
REGISTRATION NUMBER

April 24, 2001

09/830123

JC18 Rec'd PCT/PTO 24 APR 2001

Patent
Attorney's Docket No. 001560-397

# IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|---|---|---|
| In re Patent Application of | ) | |
| | ) | |
| Shigeru IIDA et al | ) | Group Art Unit: To be assigned |
| | ) | |
| Application No.: To be assigned | ) | Examiner: To be assigned |
| (National Stage of PCT International Appln. | ) | |
| No. PCT/JP00/05722 filed August 24, 2000) | ) | |
| | ) | |
| Filed: April 24, 2001 | ) | |
| | ) | |
| For: GENES ENCODING PROTEINS | ) | |
| REGULATING THE pH OF | ) | |
| VACUOLES | ) | |

## PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application on the merits, please amend the application as follows:

IN THE SPECIFICATION

Kindly replace the paragraph beginning at page 5, line 15, with the following:

-- The present invention also provides a plant in which said gene or said vector has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.--

Kindly replace the paragraph beginning at page 5, line 19, with the following:

-- The present invention also provides a cut flower of the above plant or a progeny thereof.--

Please add the paper copy of the Sequence Listing included herewith to the application, after page 19 and before the Claims on page 20.

Please renumber the pages accordingly.

## IN THE CLAIMS

Please replace claims 7, 9, and 11-14 as follows:

7. (Amended) A vector comprising the gene according to claim 1.

9. (Amended) A protein encoded by the gene according to claim 1.

11. (Amended) A plant in which the gene according to claim 1 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.

12. (Amended) A cut flower of the plant according to claim 11 or a progeny thereof having the same property as said plant.

13. (Amended) A method of regulating the pH of vacuoles comprising introducing the gene according to claim 1 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

14. (Amended) A method of controlling the flower color of a plant comprising introducing the gene according to claim 1 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

Please add new claims 15-51 as follows:

--15. A vector comprising the gene according to claim 2.

16. A vector comprising the gene according to claim 3.

17. A vector comprising the gene according to claim 5.

18. A vector comprising the gene according to claim 6.

19. A host cell transformed with the vector according to claim 15.

20. A host cell transformed with the vector according to claim 16.

21. A host cell transformed with the vector according to claim 17.

22. A host cell transformed with the vector according to claim 18.

23. A protein encoded by the gene according to claim 2.

24. A protein encoded by the gene according to claim 3.

25. A protein encoded by the gene according to claim 5.

26. A protein encoded by the gene according to claim 6.

27. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the host cell according to claim 19 and then harvesting said protein from said host cell.

28. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the host cell according to claim 20 and then harvesting said protein from said host cell.

29. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the host cell according to claim 21 and then harvesting said protein from said host cell.

30. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the host cell according to claim 22 and then harvesting said protein from said host cell.

31. A plant in which the gene according to claim 2 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.

32. A plant in which the gene according to claim 3 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.

33. A plant in which the gene according to claim 5 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.

34. A plant in which the gene according to claim 6 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.

35. A cut flower of the plant according to claim 31 or a progeny thereof having the same property as said plant.

36. A cut flower of the plant according to claim 32 or a progeny thereof having the same property as said plant.

37. A cut flower of the plant according to claim 33 or a progeny thereof having the same property as said plant.

38. A cut flower of the plant according to claim 34 or a progeny thereof having the same property as said plant.

39. A method of regulating the pH of vacuoles comprising introducing the gene according to claim 2 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

40. A method of regulating the pH of vacuoles comprising introducing the gene according to claim 3 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

41. A method of regulating the pH of vacuoles comprising introducing the gene according to claim 5 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

42. A method of regulating the pH of vacuoles comprising introducing the gene according to claim 6 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

43. A method of controlling the flower color of a plant comprising introducing the gene according to claim 2 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

44. A method of controlling the flower color of a plant comprising introducing the gene according to claim 3 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

45. A method of controlling the flower color of a plant comprising introducing the gene according to claim 5 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

46. A method of controlling the flower color of a plant comprising introducing the gene according to claim 6 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

47. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 1 in a plant or plant cells.

48. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 2 in a plant or plant cells.

49. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 3 in a plant or plant cells.

50. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 5 in a plant or plant cells.

51. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 6 in a plant or plant cells.--

## REMARKS

Prior to examination, entry of the foregoing is respectfully requested.

Claims 7, 9, and 11-14 have been amended simply to delete multiple dependencies in the claims and correct claim dependencies. Minor amendments relating to matters of form only have also been made.

New claims 15-51 have been added, directed to preferred embodiments of the invention in view of the deletion of multiple dependent claims. Support for these additional claims may be found at the very least in original claims 1-14 and at page 19, lines 12-24. No new matter has been added.

In the event that there are any questions relating to this Preliminary Amendment, or to the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney at (508) 339-3684 concerning such questions so that prosecution of this application may be expedited.

Early and favorable action in the form of a Notice of Allowance is respectfully requested and believed to be in order.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: #39,300
for Donna M. Meuth
Registration No. 36,607

P.O. Box 1404
Alexandria, Virginia 22313-1404
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Date: April 24, 2001

**Attachment to Preliminary Amendment dated April 24, 2001**

**Marked-up Copy**

**Page 5, Paragraph Beginning at Line 15**

The present invention also provides a plant in which said gene or said vector has been introduced or [an] a progeny thereof having the same property as said plant, or a tissue thereof.

**Attachment to Preliminary Amendment dated April 24, 2001**

**Marked-up Copy**

**Page 5, Paragraph Beginning at Line 19**

The present invention also provides a cut flower of the above plant or [an] a progeny thereof.

**Attachment to Preliminary Amendment dated April 24, 2001**

**Marked-up Claims 7, 9, and 11-14**

7. (Amended) A vector comprising the gene according to claim 1 [any one of the claims 1 to 6].

9. (Amended) A protein encoded by the gene according to claim 1 [any one of claims 1 to 6].

11. (Amended) A plant in which the gene according to claim 1 [any one of claims 1 to 6 or the vector according to claim 7] has been introduced or a [an] progeny thereof having the same property as said plant, or a tissue thereof.

12. (Amended) A cut flower of the plant according to claim 11 or a [an] progeny thereof having the same property as said plant.

13. (Amended) A method of regulating the pH of vacuoles comprising introducing the gene according to claim 1 [any one of claims 1 to 6 or the vector according to claim 7] into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

14. (Amended) A method of controlling the flower color of a plant [plants] comprising introducing the gene according to claim 1 [any one of claims 1 to 6 or the vector according to claim 7] into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

DESCRIPTION

# GENES ENCODING PROTEINS REGULATING THE pH OF VACUOLES

## Technical Field

The present invention relates to genes encoding proteins that regulate the pH of vacuoles, and the uses thereof.

## Background Art

In the flower industry, the development of novel or varied cultivars of flowering plants is important, and flower color is one of the most important traits of flowers. Although cultivars of various colors have been bred using conventional breeding by crossing, it is rare that a single plant species has cultivars of all colors. Thus, there is a need for the development of cultivars having a variety of colors.

The main components of flower color are a group of flavonoid compounds termed anthocyanins. It is known that a variety of anthocyanins occur in plants, and the structure of many of them have already been determined. The color of anthocyanins depends partly on their structures. Progress has been made in the study on the enzymes and genes involved in the biosynthesis of anthocyanins, and in some studies molecular biological techniques and gene introductions into plants were used to change the structure of anthocyanins, leading to changes in the color of flowers (Holton and Cornish, Plant Cell, 7:1071 (1995); Tanaka et al., Plant Cell Physiol. 39:1119 (1998)). The color of anthocyanins also depends on the pH of the aqueous solution, and the same anthocyanin may appear blue when the pH of the aqueous solution is neutral to weakly alkaline (Saito and Honda, Genda Kadaku (Chemistry Today), May 1998, pp. 25).

It is also known that since anthocyanins are present in the vacuole of the cell, the pH of vacuoles has a

great impact on the color of flowers (Holton and Cornish, Plant Cell, 7 (1995); Mol et al., Trends Plant Sci. 3:212 (1998)). For example, in morning glory (Ipomea tricolor), it is known that the reason why red-purple buds bloom into blue flowers is that the pH of vacuoles in petal epithelium rises from 6.6 to 7.7 (Yoshida et al., Nature 373:291 (1995)).

It is thought that the vacuole of plant cells is regulated by vacuolar proton-transporting ATPase and vacuolar proton-transporting pyrophosphatase (Leigh et al., The Plant Vacuole (1997), Academic Press), but the mechanism of how these proton pumps are involved in the color of flowers has not been elucidated. It was also known that a sodium ion-proton antiporter (hereinafter referred to as $Na^+$-$H^+$ antiporter) exits in plant vacuoles and that the $Na^+$-$H^+$ antiporter transports sodium ions into vacuoles, depending on the proton concentration gradient between the outside and the inside of vacuoles, whereupon protons are transported outside of vacuoles resulting a reduced proton concentration gradient.

Furthermore, the $Na^+$-$H^+$ antiporter is thought to be a protein with a molecular weight of about 170,000. However, there are many unknown factors involved in the regulation of pH of vacuoles, and the mechanism of regulating the pH of vacuoles, in particular the petal vacuoles, is uncertain (Leigh et al., The Plant Vacuole (1997), Academic Press). The pH of plant vacuoles has never been artificially raised, nor have any industrially useful traits been obtained, and its association with flower color is unknown.

It is known that the $Na^+$-$H^+$ antiporter gene, with a molecular weight of about 70,000, has been cloned from Arabidopsis, and a yeast into which this gene was introduced has acquired salt tolerance (Gaxiola et al., Proc. Natl. Acad. Sci. USA 96:1480-1485 (1999)), but it is not known how this antiporter regulates the pH of vacuoles in plant cells or how it is associated with

flower color.

On the other hand, in petunias, seven loci are known to be involved in the pH regulation of petal vacuoles, and it has been proposed that the pH of petal vacuoles increases when one of them turns homozygously recessive (van Houwelingen et al., Plant J. 13:39 (1998); Mol et al., Trends Plant Sci. 3:212 (1998)). One of them, Ph6, has already been cloned and was found to be a kind of transcription regulating factor (Chuck et al., Plant Cell 5:371 (1993)), but the actual biochemical mechanism involved in the pH regulation of vacuoles is unknown.

In morning glory (Ipomea nil), the analysis of mutants revealed that a number of loci are associated with the color and shape of leaves and flowers and that 19 of them are highly mutable (Iida et al., Shokubutsu Saibo Kogaku Series (Plant Cell Engineering Series) 5 (1996) pp. 132, Shujunsha; Iida et al., Annal. New York Acad. Sci. (1999) pp. 870). Among them, the one locus defined by the recessive mutation that results in purple flowers instead of blue flowers is termed the Purple locus (T. Hagiwara, The genetics of flower colours in Phrarbitis nil. J. Coll. Agr. Imp. Univ. Tokyo 51:241-262 (1931); Y. Imai, Analysis of flower colour in Pharbitis nil. J. Genet. 24:203-224 (1931)), and one allele of mutable mutation that results in flowers that produce blue sectors in purple petals was termed purple-mutable (pr-m) (Imai, J. Coll. Agric. Imp. Univ. Tokyo 12:479 (1934)). The gene derived from the Purple locus is termed Purple gene.

The blue portion is believed to be derived from somatic reverse mutation from the recessive purple, and germ cell revertants can also be separated. An allele produced from the reverse mutation of these revertants are termed herein Purple-revertant (Pr-r). Such a classical method of genetic analysis had been performed on this Purple gene, but the identity of the Purple gene and its association etc. with the pH regulation of petal

vacuoles were totally unknown.

It is believed that if the pH of vacuoles could be modified, for example if the pH of vacuoles could be raised, flower color could be turned blue. Representative plant species that lack blue colors include roses, chrysanthemums, carnations, gerberas and the like, which are very important cut flowers. Though the importance of modifying pH of vacuoles has been recognized, the identities of proteins that regulate the pH of petal vacuoles are unknown and therefore the isolation of genes encoding them has been in great demand.

Disclosure of the Invention

The present invention provides a gene of a protein that regulates the pH of vacuoles in plant cells, preferably a gene of a protein that transports protons in vacuoles, more preferably a $Na^+$-$H^+$ antiporter gene. By introducing the gene of the present invention into a plant and allowing it to be expressed, flower color can be controlled and, preferably, can be turned blue.

Thus, the present invention provides a gene encoding a protein that regulates the pH of vacuoles. This gene is, preferably, a gene encoding a $Na^+$-$H^+$ antiporter, for example a gene encoding a protein that has the amino acid sequence as set forth in SEQ ID NO: 2, or a gene encoding a protein that has an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or substitution with other amino acids in the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles; a gene encoding a protein that has an amino acid sequence having a identity of 20% or more with the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles; or, a gene that hybridizes to part or all of a nucleic acid having a nucleotide sequence encoding the amino acid sequence as set forth in

SEQ ID NO: 2 under a stringent condition, and that encodes a protein having an activity of regulating the pH of vacuoles.

The present invention also provides a vector comprising the above gene.

The present invention also provides a host cell transformed with the above vector.

The present invention also provides a protein encoded by the above gene.

The present invention further provides a method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the above host cell and then harvesting said protein from said host cell .

The present invention also provides a plant in which said gene or said vector has been introduced or an progeny thereof having the same property as said plant, or a tissue thereof.

The present invention also provides a cut flower of the above plant or an progeny thereof.

The present invention further provides a method of regulating the pH of vacuoles comprising introducing the above gene or the above vector into a plant or plant cells and then allowing it to be expressed.

The present invention further provides a method of controlling the flower color of plants comprising introducing the above gene or the above vector into a plant or plant cells and then allowing said gene to be expressed.

Brief Explanation of the Drawings

Fig. 1 is a drawing showing the structure of plasmid pSPB607.

Fig. 2 is a drawing showing the structure of plasmid pSPB608.

Fig. 3 is a drawing showing the structure of plasmid pINA145.

Fig. 4 is a drawing showing the structure of plasmid pINA147.

Best Mode for Carrying Out the Invention

The color of the petal of morning glory is blue when the locus Purple is dominant, and the blue petal turns purple when it is homozygously recessive. It is clear that the locus is associated with flower color but the mechanism thereof is unknown.

First, the chemical analysis of the pigments in the petal of the pr-m mutant and a revertant thereof detected no difference in the composition of the pigments. The change in flower color of the blue-colored morning glory from the reddish purple buds to the blue flowers accompanied by flowering is believed, as mentioned above, to be caused by pH changes in the vacuole of petal cells.

In the pr-m mutant, flowering is not associated with a color change to blue, and the pH of vacuoles of petal cells of flowers that bloomed was lower in the pr-m mutant than in Pr-r. Thus, the Purple gene is considered to be a gene that regulates the pH of vacuoles of petal cells during flowering and thereby controls flower color. Accordingly, using a pr-m mutant, and a revertant thereof, by the transposon display method, fragments of genomic DNA containing the Purple gene sequence specifically present in pr-m were identified and then the Purple gene was identified. Surprisingly, the Purple gene thus obtained had a homology with the $Na^+$-$H^+$ antiporter from Arabidopsis etc., and, in the pr-m mutation, a transposon had been inserted in the 5'-untranslated region the Purple gene.

As the gene of the present invention, there can be mentioned, for example, one that encodes the amino acid sequence as set forth in SEQ ID NO: 2. It is known, however, that proteins having an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or substitution with other

amino acids also retain an activity equal to that of the original protein. Thus in accordance with the present invention, a protein that has an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or substitution with other amino acids in the amino acid sequence as set forth in SEQ ID NO: 2, and a gene encoding said protein, are encompassed in the present invention as long as the protein is a protein that has an activity of regulating the pH of vacuoles.

The present invention also relates to a gene that hybridizes to the nucleotide sequence as set forth in SEQ ID NO: 1, a nucleotide sequence encoding the amino acid sequence as set forth in SEQ ID NO: 2, or a nucleotide sequence encoding part of these nucleotide sequences at a stringent condition, for example at 5 × SSC and 50°C, and that encodes a protein having an activity of regulating the pH of vacuoles. As used herein, a suitable hybridization temperature varies with the nucleotide sequence and the length of the nucleotide sequence, and when, for example, a DNA fragment comprising 18 bases encoding 6 amino acids is used as a probe, a temperature of 50°C or lower is preferred.

Genes selected, based on such hybridization, include those obtained from nature, for example from plants such as petunia and torenia, but a gene derived from sources other than plants may be used. Genes selected based on hybridization may be cDNA or genomic DNA.

The $Na^{+}$-$H^{+}$ antiporter genes form a superfamily (Debrov et al., FEBS Lett. 424:1 (1998)), and have an amino acid homology of 20% or more (Orlowski et al., J. Biol. Chem. 272:22373 (1997)).

Thus, the present invention relates to a gene encoding a protein that has an amino acid sequence with a homology of about 20% or more, preferably 50% or more, for example 60% or 70% or more, and that has an activity of regulating the pH of vacuoles.

A gene having an intact nucleotide sequence is obtained, as specifically illustrated in Examples, by, for example, screening cDNA libraries. DNA encoding a protein having a modified amino acid sequence can be synthesized by commonly used site-directed mutagenesis or the PCR method based on DNA having an intact nucleotide sequence. For example, a DNA fragment that is to be modified may be obtained by restriction enzyme treatment of the intact cDNA or genomic DNA, which is used as a template in the site-directed mutagenesis, or by the PCR method using primers in which desired mutation has been introduced to obtain a DNA fragment in which the desired modification has been introduced. Thereafter, the mutated DNA fragment may be ligated to a DNA fragment encoding another portion of the enzyme of interest.

Alternatively, in order to obtain DNA encoding a protein comprising a shortened amino acid sequence, an amino acid sequence longer than the amino acid sequence of interest, for example, DNA encoding the full-length amino acid sequence, may be cleaved with a desired restriction enzyme, and when the resultant DNA fragment was found not to encode the entire amino acid sequence of interest, a DNA fragment comprising the sequence of the lacking portion may be synthesized and ligated thereto.

The present invention is not limited to a gene encoding a protein that has an activity of regulating the pH of vacuoles derived from morning glory, but the sources may be plants, animals, or microorganisms, and all they need is to have a topology that pumps protons out of the vacuole.

By expressing the obtained gene using a gene expression system in Escherichia coli or yeast and determining the activity, it can be confirmed that the gene obtained encodes a protein that has an activity of regulating the pH of vacuoles. Furthermore, by expressing said gene, a protein, the gene product, having an activity of regulating the pH of vacuoles can be

obtained. Alternatively, a protein can also be obtained that has an activity of regulating the pH of vacuoles using an antibody against the amino acid sequence as set forth in SEQ ID NO: 2, and a protein that has an activity of regulating the pH of vacuoles derived from other organisms can be cloned using an antibody.

Thus, the present invention also relates to a recombinant vector comprising the above-mentioned gene, specifically an expression vector, and a host cell transformed with said vector. As a host, there can be used a prokaryotic or eukaryotic organism. As a prokaryotic organism, for example, there can be used such a common host as a bacterium belonging to the genus Escherichia such as Escherichia coli, a bacterium belonging to the genus Bacillus such as Bacillus subtilis, and the like. As a eukaryotic host, there can be used a lower eukaryotic organism, for example an eukaryotic microorganism such as a fungus, a yeast or a mold.

As yeast, there can be mentioned a microorganism belonging to the genus Saccharomyces such as Saccharomyces cerevisiae, and as a mold, there can be mentioned a microorganism belonging to the genus Aspergillus such as Aspergillus oryzae and Aspergillus niger, and a microorganism belonging to the genus Penicillium. Furthermore, animal cells or plant cells can be used: as animal cells, there can be used cell lines derived from mouse, hamster, monkey, human and the like. Insect cells such as silkworm cells or adult silkworms per se can also be used as hosts.

The vectors of the present invention may contain expression regulatory regions such as a promoter, a terminator, an origin of replication, and the like, depending on the type of the host into which said vector is to be introduced. As promoters for bacterial expression vectors, there can be used commonly used promoters such as trc promoter, tac promoter, lac

promoter, and the like; as promoters for yeasts, there can be used the glyceraldehyde-3-phosphate dehydrogenase promoter, PHO5 promoter, and the like; and as mold promoters, there can be used amylase promoter, trpC promoter, and the like.

As promoters for animal cell hosts, there can be used viral promoters such as SV40 early promoter, SV40 late promoter, and the like. The construction of expression vectors may be performed according to conventional methods using restriction enzymes, ligase, etc. The transformation of host cells can also be performed according to conventional methods.

Host cells transformed with the above expression vectors may be cultured, cultivated or bred, and from the culture the desired protein can be recovered and purified according to conventional methods such as filtration, centrifugation, cell disruption, gel filtration chromatography, ion exchange chromatography, and the like.

The present invention also relates to a plant or its progenies or tissues thereof of which hue of color has been controlled by introducing a gene encoding a protein that has an activity of regulating the pH of the vacuoles, specifically a $Na^+$-$H^+$ antiporter gene. They may be cut flowers in shape. Using a gene encoding a protein that has an activity of regulating the pH of vacuoles obtained by the present invention, the pumping of proton into the cytoplasm from the vacuole and the pumping of sodium ion into the vacuole can be performed, so that anthocyanins accumulated in the vacuole can be turned blue and, as a result, the flower color can be turned blue.

It is also possible to lower the pH of vacuoles by suppressing the expression of the gene of the present invention. With the state-of-the-art technology, it is possible to introduce a gene into plants, and allow the gene to be expressed in a constitutive or tissue-specific

manner, and also to suppress the expression of the gene of interest by the antisense method or the co-suppression method.

Examples of plants that can be transformed include, but not limited to, roses, chrysanthemums, carnations, snapdragons, cyclamens, orchids, lisianthus, freesias, gerberas, gladioluses, gypsophilas, kalanchoes, lilies, pelargoniumas, geraniums, petunias, torenias, tulips, rice, barley, whieat, rapeseeds, potatoes, tomatoes, poplars, bananas, eucalyptuses, sweet potatoes, soy beans, alfalfas, lupins, corns, and the like.

Examples

The present invention will now be explained in further details with reference to the following Examples. Molecular biological techniques used were performed according to Molecular Cloning (Sambrook et al., 1989), unless otherwise specified.

Example 1. Obtaining a germ cell revertant

Obtaining a germ cell revertant has already been reported (Iida et al., Shokubutsu Saibo Kogaku Series (Plant Cell Engineering Series) 5 (1996) pp. 132, Shujunsha; Iida et al., Annal. New York Acad. Sci. (1999) pp. 870; Inagaki et al., Plant Cell, 6:375 (1994); Inagaki et al., Theor. Appl. Genet. 92:499 (1996)).

Morning glory having the genotype (Pr-r/pr-m) (Iida et al., pp. 870; Inagaki et al., Plant Cell, 6:375 (1994); Inagaki et al., Theor. Appl. Genet. 92:499 (1996)) was subjected to self-fertilization and the seeds of the progeny were planted. The flowers of the self-fertilized progeny were observed to select individuals that bloom with blue flowers by back mutation. Furthermore, in this self-fertilized progeny of the germ cell revertant, it was proved whether it is homozygous or heterozygous based on whether or not isolates that bloom with purple flowers can be obtained. Those having the genotype (Pr-r/Pr-r) and (pr-m/pr-m) were selected.

Example 2. Anthocyanins in the petals of revertants

Anthocyanins contained in morning glory are mainly heavenly blue anthocyanin and several other anthocyanins (Lu et al., Phytochemistry 31:659 (1992)). When the open petals of the Pr-r/Pr-r strain and the pr-m/pr-m strain obtained in Example 1 were similarly analyzed, the anthocyanins contained in both of them were almost identical.

A cellophane tape was stuck to the front side of a petal and then peeled off to recover one layer of epithelium, from which the cell liquid was scraped with a scalpel etc., which was then centrifuged to obtain juice. The pH of the juice was measured using the Horiba B212 pH meter (Horiba Seisakusho). pH of the petal epithelium of the Pr-r/Pr-r strain was about 7.1 whereas that of the pr-m/pr-m strain was about 6.5. This result indicates that the change in flower color by mutation of purple was not due to the structure of anthocyanins but to the change of vacuolar pH.

Example 3. Isolation of a genome fragment specifically present in pr-m

For the isolation of a gene, the transposon display method (Frey et al., Plant J. 13:717 (1998); Van den Broeck et al., Plant J. 13:121 (1998)) or a similar method (Dosho et al., Shokubutsu Saibo Kogaku Series (Plant Cell Engineering Series) 7 (1997) pp. 144, Shujunsha) was used to search for DNA bands that were present in the pr-m/pr-m strain and the Pr-w/pr-m strain but not in the Pr-r/Pr-r strain or in the wild strain. Since Tpn1-related transposon is thought to be mainly associated with mutability in morning glory, special note was given to the Tpn1-related transposon.

Specifically, chromosomal DNA was extracted from the pr-m/pr-m strain, and 125 ng of it was digested with MseI in 20 μl. To the digested DNA was added 80 pmole of MseI adaptor (obtained by annealing 5'-GACGATGAGTCCTGAG-3' (SEQ ID NO: 3) and 5'-TACTCAGGACTCAT-3' (SEQ ID NO: 4))

in 25 μl at 20°C for 2 hours. After keeping it at 75°C for 10 minutes, it was stored at -20°C. After diluting this ten-fold, 2 μl was used as a template, which was PCR-amplified using 4.8 pmole of TIR primer (5'-TGTGCATTTTTCTTGTAGTG-3' (SEQ ID NO: 5), this includes the inverted terminal repeat of the transposon Tpn1) and 4.8 pmole of MseI primer (5'-GATGAGTCCTGAGTAA-3') (SEQ ID NO: 6) in 20 μl.

PCR was performed with Taq polymerase (Takara Shuzo) for 20 cycles with one cycle comprising 94°C for 0.5 minute, 56°C for 1 minute, and 72°C for 1 minute, and the volume was diluted ten-fold. Two μl of it was used as a template in a PCR using 4.8 pmole of TIR+N primer (5'-TGTGCATTTTTCTTGTAGN-3' (SEQ ID NO: 7) N=A, C, G or T. Four different species were synthesized instead of a mixture) and 4.8 pmole of MseI+N primer (5'-GATGAGTCCTGAGTAAN-3' (SEQ ID NO: 8) N=A, C, G or T. Four different species were synthesized instead of a mixture. The 5'-end was labeled with fluorescein (using Amersham Pharmacia Biotek, Vistra fluorescence 5'-oligo labeling kit)) in 20 μl.

Reactions were performed for combinations of primers to a total of 16 reactions. PCR was performed for 13 cycles with one cycle comprising 94°C for 0.5 minute, 65°C (with a decrement of 0.7°C for each cycle) for 1 minute, and 72°C for 1 minute, and further for 13 cycles with one cycle comprising 94°C for 0.5 minute, 56°C for 1 minute, and 72°C for 1 minute. A similar procedure was performed for chromosomal DNA obtained from the Pr-r/Pr-r strain, subjected to electrophoresis using a sequence gel of the DNA Sequencer 377 (PE Biosystems Japan), and the bands were detected using FMBIOII (Takara Shuzo).

When bands derived from the Pr-r/Pr-r strain and the pr-m/pr-m strain were compared, an about 130 bp DNA fragment was specifically expressed in the strain having

pr-m. The 130 bp DNA fragment was recovered, and amplified by PCR (for 30 cycles with one cycle comprising 94°C for 0.5 minute, 56°C for 1 minute, and 72°C for 1 minute) using 20 pmole TIR primer and 20 pmole MseI primer, which was then subcloned into the pGEM-T vector (Promega Corporation), and then the nucleotide sequence was determined. The sequence was 5'-TGAGCATTTTTCTTGTAGTG CTGAGATTTTCCTCCATTTGTCTGAAGCTCTTCATCCTTCAACAC TACCCCCACATCTCACCTTTCAAG GTCCAATCTTTATCATTCATCT TTACTCAGGACTCATCGTC-3' (SEQ ID NO: 9) (the single-underlined portion corresponds to a used primer, the double-underlined portion corresponds to an exon, and the rest corresponds to an intron). After the sequence as set forth in SEQ ID NO: 9 was used as a probe in Northern analysis, a transcription product of about 2.3 kb was found in the bud of morning glory having Pr-r, but a corresponding transcription product was not found in the pr-m/pr-m strain. Thus, it can be seen that this 2.3 kb transcription product corresponds to the Purple gene.

Example 4. Isolation of cDNA

About 6 million clones of a cDNA library (Inagaki et al., Plant Cell 6:375 (1994)) derived from the wild strain morning glory (Pr-w/Pr-w) were screened using the 130 bp DNA fragment as a probe, with a result that two positive clones were obtained. One of these clones had a 2237 bp cDNA, among which a 1626 bp-long open reading frame was observed (SEQ ID NO: 1). The predicted amino acid sequence had an identity of 29.3% and 73.4% with the $Na^+$-$H^+$ antiporter of yeast and Arabidopsis, respectively (Nhx1 and AtNhx1, respectively, Gaxiola et al., Proc. Natl. Acad. Sci. USA 96:1480-1485 (1999)).

The result revealed that the Purple gene of morning glory encodes a $Na^+$-$H^+$ antiporter. Incidentally, although the $Na^+$-$H^+$ antiporter obtained from Arabidopsis is attracting attention as a protein that gives salt resistance to yeast, this is the first time that an association of the $Na^+$-$H^+$ antiporter with flower color

was observed.

Example 5. Complementation experiment of yeast $Na^{+}$-$H^{+}$ antiporter

The predicted amino acid sequence encoded by the Purple gene of morning glory has a homology with those of the $Na^{+}$-$H^{+}$ antiporters of yeast and Arabidopsis. Thus, in order to confirm whether the Purple gene product of morning glory can function as a $Na^{+}$-$H^{+}$ antiporter protein, a complementation experiment was performed using a yeast $Na^{+}$-$H^{+}$ antiporter mutant.

First, the following two DNA fragments were synthesized:

CBSC1-Linker (22 mer) 5'-CGA TAG ATC TGG GGG TCG ACA T-3' (SEQ ID NO: 12)

CSBD2-Linker (22 mer) 5'-CGA TGT CGA CCC CCA GAT CTA T-3' (SEQ ID NO: 13)

From these two fragments, a linker having restriction enzyme sites ClaI-BglII-SalI-ClaI is formed. A plasmid pINA145 (Fig. 3) was constructed by inserting the above linker according to a standard method into the ClaI site of the pYES2 vector (Invitrogen Corporation) so that the BglII site is located at the URA3 gene side. A plasmid pINA147 (Fig. 4) was constructed by ligating a 2 kb DNA fragment obtained by digesting plasmid pJJ250 (Jones and Prakash, Yeast 6:363-366 (1990)) with BamHI and SalI to plasmid pINA145 digested with BglII and SalI. Plasmid pIAN151 was constructed by ligating Purple cDNA thereto under the control of the GAL 1 promoter of plasmid pINA147. pINA147 and pIAN151 were transformed respectively to the yeast R101 strain which is a mutant strain of the $Na^{+}$-$H^{+}$ antiporter. Due to the mutation of the $Na^{+}$-$H^{+}$ antiporter, the yeast R101 strain cannot grow on a 400 mM NaCl-added APG medium (Nass et al., J. Biol. Chem. 272:26145 (1997); Gaxiola et al., 96:1480-1485 (1999)). The pINA147-transformed R101 strain could not grow either, and only the pIAN151-transformed R101 strain could grow on the 400 mM NaCl-added APG medium. The

result has shown that the gene product of the morning glory Purple gene has the $Na^{+}$-$H^{+}$ antiporter function.

Example 6. Construction of an expression vector in plants

With 10 ng of morning glory Purple cDNA as template, PCR was performed using synthetic primers PR-5 (5'-GGGATCCAACAAAAATGGCTGTCGGG-3') (SEQ ID NO: 10) and PR-3 (5'-GGGTCGACTAAGCATCAAAACATAGAGCC-3') (SEQ ID NO: 11). The polymerase used was Taq polymerase (Toyoboseki), and the reaction was performed, after reaction at 95°C for 45 seconds, for 25 cycles with one cycle comprising 95°C for 45 seconds, 50°C for 45 seconds, and 72°C for 45 seconds, and then further reacted at 72°C for 10 minutes. An about 1.6 kb DNA fragment obtained was ligated to pCR2.1-Topo (Clontech) to make pCR-purple. It was confirmed that there were no errors due to PCR in the nucleotide sequence of Purple cDNA on this plasmid.

pBE2113-GUS (Mitsuhara et al., Plant Cell Physiol. 37:49 (1996)) was digested with SacI and blunt-ended. Then a XhoI linker (Toyoboseki) was inserted thereto, and the plasmid obtained was termed pBE2113-GUSx. This was digested with EcoRI and HindIII to obtain an about 2.7 kb DNA fragment, which was ligated to the HindIII and EcoRI digest of pBinPLUS, and the plasmid obtained was termed pBEXP.

On the other hand, an about 1.2 kb DNA fragment obtained by digesting pCGP484 (Kohyo (National Publication of Translated Version) No. 8-511683) with HindIII and XbaI, an about 1.6 kb DNA fragment obtained by digesting pCR-purple with XbaI and SalI, and an about 13 kb DNA fragment obtained by digesting pBEXP with HindIII and XhoI were ligated to obtain pSPB607 (Fig. 1). This plasmid is a binary vector for use in the Agrobacterium-mediated transformation of plants, and on this plasmid Purple cDNA is under the control of a chalcone synthase promoter derived from snapdragon and a nopaline synthase terminator derived from Agrobacterium.

An about 0.8 kb DNA fragment obtained by digesting pCGP669 (Kohyo (National Publication of Translated Version) No. 8-511683) with HindIII and BamHI, an about 1.6 kb DNA fragment obtained by digesting pCR-purple with BamHI and SalI, and an about 13 kb DNA fragment obtained by digesting pBEXP with HindIII and XhoI were ligated to obtain pSPB608 (Fig. 2). This plasmid is a binary vector for use in the Agrobacterium-mediated transformation of plants, and on this plasmid Purple cDNA is under the control of a chalcone synthase promoter derived from petunia and a nopaline synthase terminator derived from Agrobacterium.

By transforming plants using the expression vectors thus obtained, the pH of vacuoles can be regulated and thereby flower color can be controlled.

<u>Example 7.</u> <u>Isolation of a homologs of the Purple gene</u>

cDNA libraries derived from the petals of petunia (Petunia hybrida cv. Old Glory Blue), Nierembergia (Nierembergia hybrida cv. NB17), and Torenia (Torenia hybrida cv. Summerwave Blue) were each constructed using the cDNA synthesis kit (Stratagene, USA). The method of construction was as recommended by the manufacturer. About 200,000 clones each were screened according to a standard method. For washing the membrane, an aqueous solution of 5 × SSC and 0.1% SDS was used and the incubation was performed three times at 50°C for 10 minutes. Among the positive clones obtained, the nucleotide sequence of the longest clone was determined for each clone. The nucleotide sequence of the clone of Petunia and the corresponding amino acid sequence are shown in SEQ ID NO: 14 and 15, the nucleotide sequence of the clone of Nierembergia and the corresponding amino acid sequence are shown in SEQ ID NO: 16 and 17, and the nucleotide sequence of the clone of Torenia and the corresponding amino acid sequence are shown in SEQ ID NO: 18 and 19. Homologs of the Purple gene of Petunia, Nierembergia, and Torenia had an identity on the amino

acid level of 75%, 76%, and 71%, respectively, with the morning glory Purple gene.

Since the amino acid sequence of the $Na^+$-$H^+$ antiporter encoded by the morning glory Purple gene and that of the $Na^+$-$H^+$ antiporter encoded by Arabidopsis AtNhx 1 are about 73% identical, the homologs of the Purple gene of Petunia, Nierembergia, and Torenia obtained are judged to encode the $Na^+$-$H^+$ antiporter.

<u>Example 8.</u> <u>Isolation of the clone of morning glory Purple chromosome</u>

After chromosomal DNAs of a mutant morning glory (pr-m/pr-m) and a revertant morning glory (Pr-r/Pr-r) were cleaved with BglII, they were electrophoresed on a 0.8% agarose gel, and were subjected to genomic Southern analysis with cDNA of morning glory Purple as a probe. As a result, an about 7.5 kb band that was not present in the mutant morning glory was detected in the revertant morning glory.

After 50 μg of chromosomal DNA of the wild type morning glory (Pr-w/Pr-w, the KKZSK2 strain) was digested with BglII, it was electrophoresed on a 0.8% agarose gel. An about 7-9 kb fragmently was recovered, from which DNA was extracted using the GENECLEAN III KIT (B10101). This DNA was ligated to the λ Zap express vector (Stratagene, USA), which was screened with cDNA of morning glory Purple as a probe. The determination of nucleotide sequences of positive clones obtained revealed that, on this about 7.5 kb DNA fragment, there was a region from about 6.3 kb upstream of the Purple promoter to midway in exon 3. For this sequence, a sequence up to the initiation codon of the Purple gene is shown in SEQ ID NO: 20.

It has been demonstrated that the expression of the Purple gene is strongly induced only at about 24 hours before the flowering of morning glory, and that the expression of the Purple gene is suppressed by insertion

of a transposon into the 5'-untranslated region. From this, it is clear that the promoter region of the Purple gene obtained contains a factor needed for the expression of the Purple gene in a developmental stage-specific and organ-specific manner in the petals of morning glory. By placing the gene of interest downstream of this promoter region, the expression of the gene of interest can be regulated in a developmental stage-specific and organ-specific manner.

## Industrial Applicability

The gene obtained in the present invention was found, for the first time, to be involved in controlling the pH of vacuoles and flower color. By expressing the gene of the present invention on the flower petals, the pH of vacuoles can be increased and thereby the flower color can be turned blue. Furthermore, by suppressing the expression of the gene of the present invention, the pH of vacuoles can be lowered and thereby flower color can be turned red. As the gene encoding a protein that regulates the pH of vacuoles, there can be used not only those derived from morning glory obtained in the present invention but also similar genes derived from other organisms.

CLAIMS

1. A gene encoding a protein that has an activity of regulating the pH of vacuoles in plant cells.

2. A gene encoding a protein that has the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles in plant cells.

3. A gene encoding a protein that has an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or substitution with other amino acids in the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles.

4. The gene according to claim 1 encoding a protein that has an amino acid sequence having a identity of 20% or more with the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles.

5. The gene according to claim 1 encoding a protein that has an amino acid sequence having a identity of 70% or more with the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles.

6. The gene according to claim 1 that hybridizes to a part or all of a nucleic acid having a nucleotide sequence encoding the amino acid sequence as set forth in SEQ ID NO: 2 under a stringent condition, and that encodes a protein having an activity of regulating the pH of vacuoles.

7. A vector comprising the gene according to any one of the claims 1 to 6.

8. A host cell transformed with the vector according to claim 7.

9. A protein encoded by the gene according to any one of the claims 1 to 6.

10. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method

comprising culturing or growing the host cell according to claim 8 and then harvesting said protein from said host cell.

11. A plant in which the gene according to any one of the claims 1 to 6 or the vector according to claim 7 has been introduced or an progeny thereof having the same property as said plant, or a tissue thereof.

12. A cut flower of the plant according to claim 11 or an progeny thereof having the same property as said plant.

13. A method of regulating the pH of vacuoles comprising introducing the gene according to any one of the claims 1 to 6 or the vector according to claim 7 into a plant or plant cells and then allowing said gene to be expressed.

14. A method of controlling the flower color of plants comprising introducing the gene according to any one of the claims 1 to 6 or the vector according to claim 7 into a plant or plant cells and then allowing said gene to be expressed.

09/830123

Fig.1

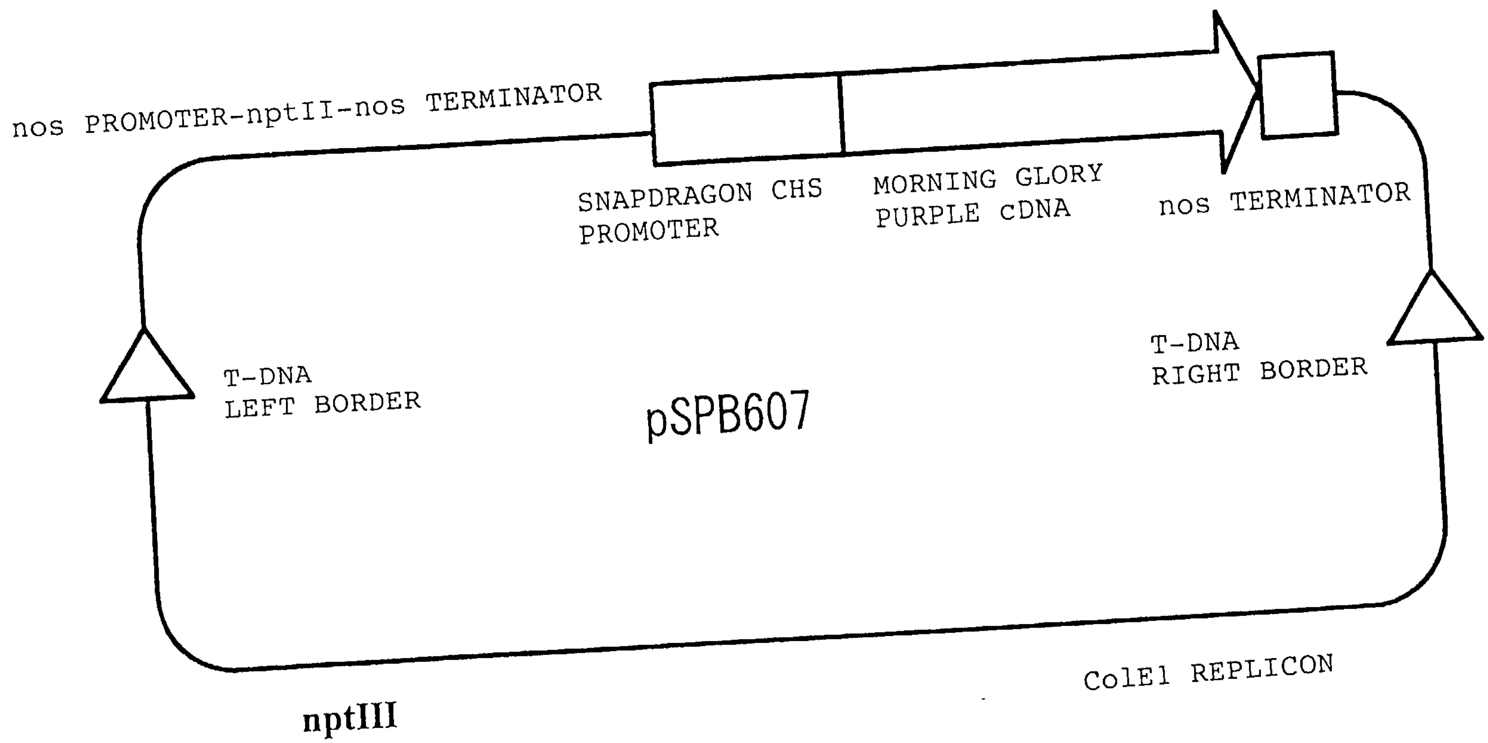


Fig. 2

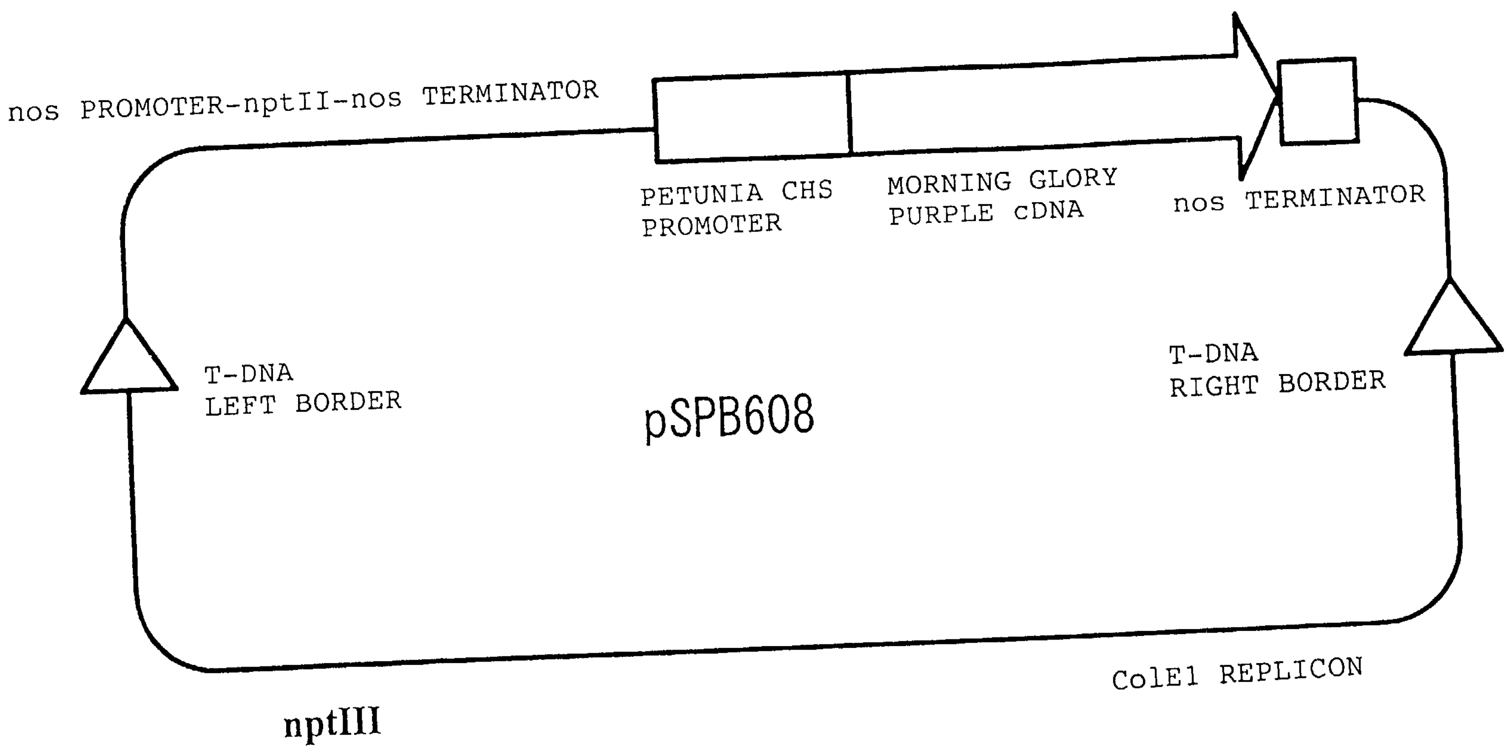
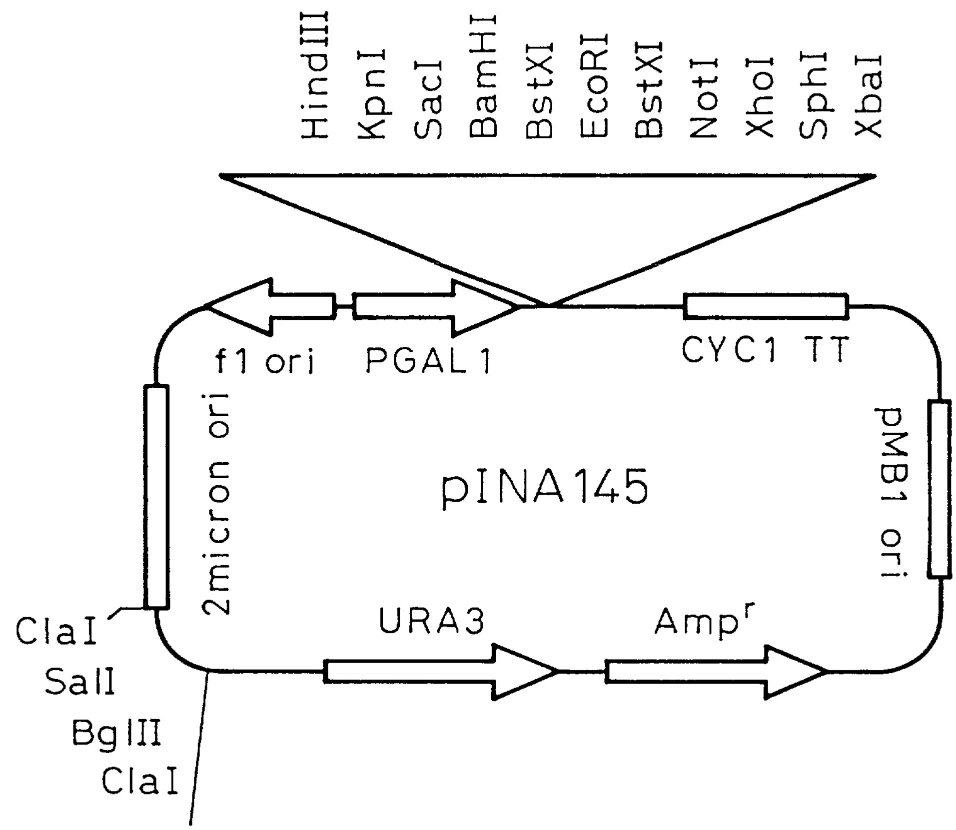

nos PROMOTER-nptII-nos TERMINATOR
PETUNIA CHS PROMOTER
MORNING GLORY PURPLE cDNA
nos TERMINATOR
T-DNA LEFT BORDER
pSPB608
T-DNA RIGHT BORDER
nptIII
ColE1 REPLICON

Fig.3


HindIII
KpnI
SacI
BamHI
BstXI
EcoRI
BstXI
NotI
XhoI
SphI
XbaI
f1 ori
PGAL1
CYC1 TT
pMB1 ori
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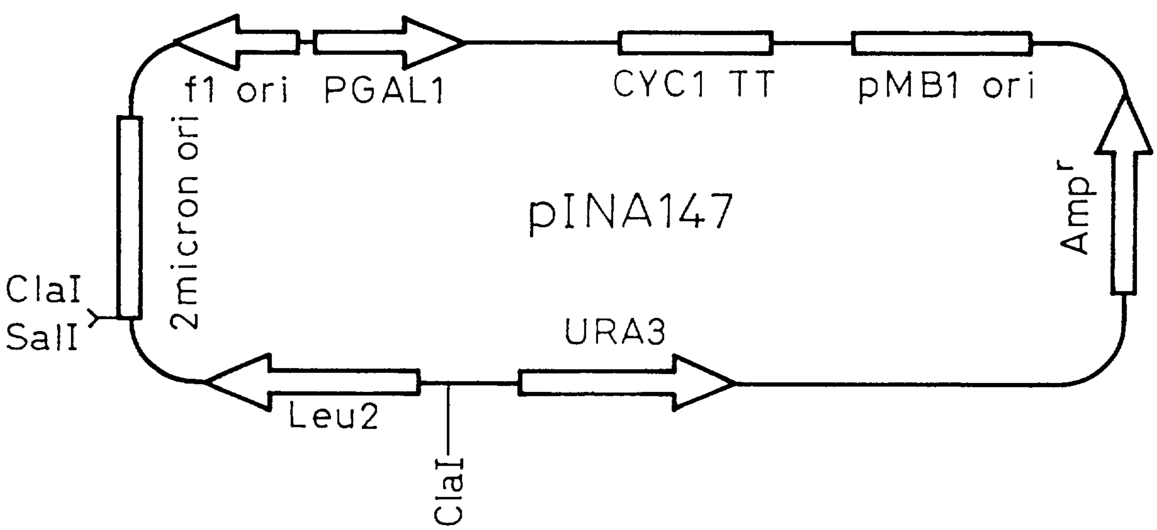
pINA145
URA3
Ampr
ClaI
SalI
BglII
ClaI

Fig.4


f1 ori
PGAL1
CYC1 TT
pMB1 ori
2micron ori
pINA147
Ampr
ClaI
SalI
URA3
Leu2
ClaI

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# Declaration and Power of Attorney For Patent Application

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## Japanese Language Declaration

## 日本語宣言書

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As a below named inventor, I hereby declare that:

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My residence, post office address and citizenship are as stated next to my name.

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者であると（下記の名称が複数の場合）信じています。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

GENES ENCODING PROTEINS REGULATING THE pH OF VACUOLES

上記発明の明細書（下記の欄でx印がついていない場合は、本書に添付）は、

the specification of which is attached hereto unless the following box is checked:

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☐ was filed on August 24, 2000 as United States Application Number or PCT International Application Number PCT/JP00/05722 and was amended on ＿＿＿＿＿ (if applicable).

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Prior Foreign Application(s)
外国での先行出願

| (Number) (番号) | (Country) (国名) | (Day/Month/Year Filed) (出願年月日) | Priority Not Claimed 優先権主張なし |
|---|---|---|---|
| 11-236800(Pat.Appln.) | Japan | 24/August/1999 | ☐ |
| | | | ☐ |

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I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

| (Application No.) (出願番号) | (Filing Date) (出願日) | (Application No.) (出願番号) | (Filing Date) (出願日) |
|---|---|---|---|
| | | | |

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I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s), or 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of application.

| (Application No.) (出願番号) | (Filing Date) (出願日) | (Status: Patented, Pending, Abandoned) (現況：特許許可済、係属中、放棄済) |
|---|---|---|
| | | |
| | | |

私は、私自身の知識に基ずいて本宣言書中で私が行なう表明が真実であり、かつ私の入手した情報と私の信じるところに基ずく表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第18編第1001条に基ずき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行なえば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

# Japanese Language Declaration
## (日本語宣言書)

委任状： 私は下記の発明者として、本出願に関する一切の手続きを米特許商標局に対して遂行する弁理士または代理人として、下記の者を指名いたします。（弁護士、または代理人の氏名及び登録番号を明記のこと）

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith *(list name and registration number)*

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| Robert S. Swecker | 19,885 | James A. LaBarre | 28,632 | Richard J. McGrath | 29,195 |
| Platon N. Mandros | 22,124 | E. Joseph Gess | 28,510 | Matthew L. Schneider | 32,814 |
| Benton S. Duffett, Jr. | 22,030 | R. Danny Huntington | 27,903 | Michael G. Savage | 32,596 |
| Joseph R. Magnone | 24,239 | Eric H. Weisblatt | 30,505 | Gerald F. Swiss | 30,113 |
| Norman H. Stepno | ~~22,716~~ | James W. Peterson | 26,057 | Michael J. Ure | ~~33,089~~ |
| Ronald L. Grudziecki | ~~24,970~~ | Teresa Stanek Rea | 30,427 | Charles F. Wieland III | ~~33,096~~ |
| Frederick G. Michaud, Jr. | ~~26,003~~ | Robert E. Krebs | 25,885 | Bruce T. Wieder | 33,815 |
| Alan E. Kopecki | 25,813 | William C. Rowland | 30,888 | Todd R. Walters | ~~34,040~~ |
| Regis E. Slutter | 26,999 | T. Gene Dillahunty | 25,423 | | |
| Samuel C. Miller, III | 27,360 | Patrick C. Keane | 32,858 | | |
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| 発明者の署名 日付 | Inventor's signature Date<br>[signature] April 16, 2001 |
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| 国籍 | Citizenship<br>Japanese |
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| | Okazaki-shi, Aichi 444-0874, Japan |
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| 第二共同発明者 日付 | Second inventor's signature Date<br>田中幸子 April 16, 2001 |
| 住所 | Residence<br>Okazaki-shi, Aichi, Japan JPX ST |
| 国籍 | Citizenship<br>Japanese |
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| | Aichi 444-0802, Japan ST |

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| 第三共同発明者 | 日付 | Third inventor's signature<br>稲垣善秀 | Date<br>April 16, 2001 |
| 住　所 | | Residence<br>Okazaki-shi, ~~Aichi,~~ Japan | JPX |
| 国　籍 | | Citizenship<br>Japanese | |
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| | | Okazaki-shi, Aichi 444-0851, Japan | |
| 第四共同発明者 | | Full name of fourth joint inventor, if any | |
| 第四共同発明者 | 日付 | Fourth inventor's signature | Date |
| 住　所 | | Residence | |
| 国　籍 | | Citizenship | |
| 私書箱 | | Post Office Address | |
| | | | |

| | | | |
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| 第五共同発明者 | | Full name of fifth joint inventor, if any | |
| 第五共同発明者 | 日付 | Fifth inventor's signature | Date |
| 住　所 | | Residence | |
| 国　籍 | | Citizenship | |
| 私書箱 | | Post Office Address | |
| | | | |
| 第六共同発明者 | | Full name of sixth joint inventor, if any | |
| 第六共同発明者 | 日付 | Sixth inventor's signature | Date |
| 住　所 | | Residence | |
| 国　籍 | | Citizenship | |
| 私書箱 | | Post Office Address | |
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09/830123

JC18 Rec'd PCT/PTO 24 APR 2001

SEQUENCE LISTING

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Cys Ala Cys Ile Val Leu Gly His Leu Leu Glu Glu Asn Arg Trp Val
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135 140 145

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Phe Asn Ala Ile Gln Asn Phe Asp Leu Ser His Ile Asp Thr Gly Lys
195 200 205 210

gct atg gaa tta gtt gga aac ttt cta tac ttg ttt gcc tca agc act 1029
Ala Met Glu Leu Val Gly Asn Phe Leu Tyr Leu Phe Ala Ser Ser Thr
215 220 225

gcc cta gga gtt gct gct ggc cta ctg agc gcc tat att att aaa aaa 1077
Ala Leu Gly Val Ala Ala Gly Leu Leu Ser Ala Tyr Ile Ile Lys Lys
230 235 240

ctc tac ttt gga agg cac tca act gac cgt gag gtt gct ata atg ata 1125
Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile
245 250 255

ctc atg gct tac cta tct tac atg ctt gct gaa tta ttc tat tta agt 1173
Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr Leu Ser
260 265 270

gca atc ctc act gtg ttt ttc tct ggg atc gtg atg tct cac tac acc 1221
Ala Ile Leu Thr Val Phe Phe Ser Gly Ile Val Met Ser His Tyr Thr
275 280 285 290
tgg cat aat gtg act gag agc tcg aga gtc act acc aag cac act ttt 1269
Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His Thr Phe
295 300 305
gct aca tta tca ttt att gct gaa ata ttc ata ttc ctt tat gtt ggt 1317
Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr Val Gly
310 315 320
atg gat gct ttg gac att gag aag tgg aag ttt gta agc gac agc cct 1365
Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp Ser Pro
325 330 335
gga ata tca gtt cag gtt agc tca ata ttg ctg ggt ctt gtt ttg gtt 1413
Gly Ile Ser Val Gln Val Ser Ser Ile Leu Leu Gly Leu Val Leu Val
340 345 350
gga aga gca gca ttt gtt ttc cca ttg tca ttc ttg tcc aac ttg acc 1461
Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu Thr
355 360 365 370
aag aaa act cca gag gcg aaa att agt ttt aac cag cag gtt aca ata 1509
Lys Lys Thr Pro Glu Ala Lys Ile Ser Phe Asn Gln Gln Val Thr Ile
375 380 385
tgg tgg gct gga ctt atg aga ggt gcc gtt tct atg gcc ctt gct tat 1557
Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu Ala Tyr
390 395 400
aat cag ttt acc agg gga ggt cat act cag tta cgc gca aat gca ata 1605
Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn Ala Ile
405 410 415
atg atc aca agt act atc act gtt gtc ctt ttc agc aca gtc gtg ttt 1653
Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val Val Phe
420 425 430
ggg ttg atg aca aaa cct ttg att aga ata ttg cta ccc tca cac aaa 1701
Gly Leu Met Thr Lys Pro Leu Ile Arg Ile Leu Leu Pro Ser His Lys
435 440 445 450
cac ttg agc aga atg atc tct tct gaa cca acg acc cca aaa tcc ttc 1749
His Leu Ser Arg Met Ile Ser Ser Glu Pro Thr Thr Pro Lys Ser Phe
455 460 465

att gtg cca ctt ctt gac agc aca caa gac tca gaa gct gat ctg gaa 1797
Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp Leu Glu
470 475 480

cgc cat gta ccc cgt ccc cac agt ttg cgg atg ctc ctt tca acc cca 1845
Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser Thr Pro
485 490 495

tct cat aca gtg cat tat tac tgg aga aag ttt gac aat gca ttc atg 1893
Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala Phe Met
500 505 510

cgt cca gtt ttc ggt gga cga ggt ttt gta cct ttt gct cca gga tca 1941
Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Ala Pro Gly Ser
515 520 525 530

ccg aca gac cca gtt ggt gga aat ttg caa tgatggagat acagattgca 1991
Pro Thr Asp Pro Val Gly Gly Asn Leu Gln
535 540

aaaagtggtc ttggtgaggg aagagggcag tttttggta atgaggttcc gttttcttta 2051
atgttaatag caagtgtggt taaaaagggg ttgtctagtt tataggtttt gcagatctca 2111
agtatattca tttgggtgat catgttttca gctcagttat tgcttttggt cattgctgac 2171
catcaatttc tgtggggaat tcctataggt tttctcccta acagttcttt tcttcatctt 2231
tttgcaattt atcgaaacac caaatgggtg tatattctgt aagcttgtgg catagctagc 2291
ttaattgtct tgtaaaattt cctacaggtt agagattggt tcttgatatg tagatttcat 2351
atgattgtaa cattcccatt tctcagaaaa gaaactataa tataaaattt ctggtggctg 2411
tcgcccgtgc tc 2423

<210> 15
<211> 540
<212> PRT
<213> Petunia hybrida

<223> Amino acid sequence of protein regulating the pH of vacuoles

<400> 15
Met Ala Phe Asp Phe Gly Thr Leu Leu Gly Asn Val Asp Arg Leu Ser
5 10 15

Thr Ser Asp His Gln Ser Val Val Ser Ile Asn Leu Phe Val Ala Leu
20 25 30
Ile Cys Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp
35 40 45
Met Asn Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Ile
50 55 60
Val Ile Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe
65 70 75 80
Ser Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn
85 90 95
Ala Gly Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr
100 105 110
Ile Met Leu Phe Gly Ala Leu Gly Thr Leu Ile Ser Phe Ile Ile Ile
115 120 125
Ser Leu Gly Ala Ile Gly Ile Phe Lys Lys Met Asn Ile Gly Ser Leu
130 135 140
Glu Ile Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ser Ala Thr Asp
145 150 155 160
Ser Val Cys Thr Leu Gln Val Leu Asn Gln Asp Glu Thr Pro Leu Leu
165 170 175
Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val
180 185 190
Val Leu Phe Asn Ala Ile Gln Asn Phe Asp Leu Ser His Ile Asp Thr
195 200 205
Gly Lys Ala Met Glu Leu Val Gly Asn Phe Leu Tyr Leu Phe Ala Ser
210 215 220
Ser Thr Ala Leu Gly Val Ala Ala Gly Leu Leu Ser Ala Tyr Ile Ile
225 230 235 240
Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile
245 250 255
Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr
260 265 270
Leu Ser Ala Ile Leu Thr Val Phe Phe Ser Gly Ile Val Met Ser His
275 280 285
Tyr Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His
290 295 300

Thr Phe Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr
305                 310                 315                 320

Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp
                325                 330                 335

Ser Pro Gly Ile Ser Val Gln Val Ser Ser Ile Leu Leu Gly Leu Val
            340                 345                 350

Leu Val Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn
        355                 360                 365

Leu Thr Lys Lys Thr Pro Glu Ala Lys Ile Ser Phe Asn Gln Gln Val
    370                 375                 380

Thr Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu
385                 390                 395                 400

Ala Tyr Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn
                405                 410                 415

Ala Ile Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val
            420                 425                 430

Val Phe Gly Leu Met Thr Lys Pro Leu Ile Arg Ile Leu Leu Pro Ser
        435                 440                 445

His Lys His Leu Ser Arg Met Ile Ser Ser Glu Pro Thr Thr Pro Lys
    450                 455                 460

Ser Phe Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp
465                 470                 475                 480

Leu Glu Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser
                485                 490                 495

Thr Pro Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala
            500                 505                 510

Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Ala Pro
        515                 520                 525

Gly Ser Pro Thr Asp Pro Val Gly Gly Asn Leu Gln
    530                 535                 540

<210> 16

<211> 2553

<212> DNA

<213> Nierembergia hybrida

<223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles

<400> 16

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aattattatt atttctctcc aactctcatt tctcagtttg ttgtgacttt ttcagagctt    60
gaagttcagt taattcattt tccaatatat tgattgtttt catttgagcg cgagaggatt   120
tcgtcttctc aatctgcttt caaatccttt ttgtttgtga tattcgatat tattcactca   180
gtttacctta atatttcctc gcactttctg aattcgagtg ctttgaagtg tgttggattt   240
cgaaaagcgg aagaaaattc agcaaaaacg ctgttgctga atttgcagca gtttgagttt   300
ttgctaaata gctaagatct gattgaattt ttcactggtg cttataggga aattcgacgt   360
cgttttgact gcaatatttg tccgtgattc ggactttgtt gaaattttgc tatttgaaat   420
ttgaatgtaa ggttgtcata gctttgccac tcggaaatac agtcagtgag aaagaaaaaa   480
aactgtgtag tgtttttttcc acaagtattt ggtgaattga ggttcttgaa atg gcg      536
                                                         Met Ala

ttt gac ttt ggg act ctg ctg gga aag atg aac aac tta aca act tct      584
Phe Asp Phe Gly Thr Leu Leu Gly Lys Met Asn Asn Leu Thr Thr Ser
            5                   10                  15

gat cat caa tca gtg gtg tcg gta aac ttg ttt gtt gca ctt att tgc      632
Asp His Gln Ser Val Val Ser Val Asn Leu Phe Val Ala Leu Ile Cys
        20                  25                  30

gcg tgt att gtg atc ggt cat tta ttg gag gaa aac aga tgg atg aat      680
Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn
 35                 40                  45                  50

gag tcc ata act gcc ctt gtg att ggt agt tgc act gga gtc atc att      728
Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Val Ile Ile
                55                  60                  65

cta cta ata agt gga gga aag aac tca cat att tta gtg ttc agc gaa      776
Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe Ser Glu
            70                  75                  80

gat ctt ttc ttc att tac ctt ctt cca ccg atc att ttt aat gct ggg      824
Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala Gly
        85                  90                  95
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ttc cag gtg aaa aag aaa tca ttc ttc cgc aat ttc agt act atc atg 872
Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr Ile Met
100 105 110

ctc ttt ggg gca gtt ggc acc ttg ata tcg ttc att att ata tca gcg 920
Leu Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile Ser Ala
115 120 125 130

ggt gct att ggc att ttc aag aaa atg gat att gga cac ctt gaa att 968
Gly Ala Ile Gly Ile Phe Lys Lys Met Asp Ile Gly His Leu Glu Ile
135 140 145

gga gat tac ctt gca att gga gca atc ttt gct gca aca gat tct gta 1016
Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser Val
150 155 160

tgc acc tta caa gtg ctt aat cag gaa gaa aca ccg tta ttg tac agt 1064
Cys Thr Leu Gln Val Leu Asn Gln Glu Glu Thr Pro Leu Leu Tyr Ser
165 170 175

cta gtg ttt gga gaa ggt gtt gtg aat gat gcc aca tct gta gtg ctg 1112
Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val Leu
180 185 190

ttc aat gct gtc cag aac ttt gac tta tct cat atc agc aca ggc aaa 1160
Phe Asn Ala Val Gln Asn Phe Asp Leu Ser His Ile Ser Thr Gly Lys
195 200 205 210

gct ctg caa tta att gga aac ttt cta tac ttg ttt gcc tcg agc act 1208
Ala Leu Gln Leu Ile Gly Asn Phe Leu Tyr Leu Phe Ala Ser Ser Thr
215 220 225

ttc cta ggg gtt gct gtt ggc cta cta agt gcc ttt ata att aag aaa 1256
Phe Leu Gly Val Ala Val Gly Leu Leu Ser Ala Phe Ile Ile Lys Lys
230 235 240

ctc tac ttt gga agg cac tcg act gat cgt gag gtt gct ata atg ata 1304
Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile
245 250 255

ctc atg gcg tac cta tca tac atg ctt gct gaa tta ttc tat tta agt 1352
Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr Leu Ser
260 265 270

gga atc ctc act gtg ttt ttc tgt ggg atc gtg atg tct cac tat acc 1400
Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr Thr
275 280 285 290

tgg cat aat gtg act gag agc tca aga gtc act acc aag cac acg ttt 1448
Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His Thr Phe
295 300 305

gct aca tta tca ttt att gct gaa ata ttc ata ttc ctt tat gtt ggt 1496
Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr Val Gly
310 315 320

atg gat gct ttg gac att gag aag tgg aag ttt gta agc gac agc ccc 1544
Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp Ser Pro
325 330 335

gga aca tca att aag gtc agc tca att ctg cta ggt ctt gtt ttg gtt 1592
Gly Thr Ser Ile Lys Val Ser Ser Ile Leu Leu Gly Leu Val Leu Val
340 345 350

gga agg gga gcc ttt gtt ttc ccc ttg tca ttc ttg tcc aac ttg acc 1640
Gly Arg Gly Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu Thr
355 360 365 370

aag aaa aat cct gag gac aag att agc ttt aac cag cag gtt aca ata 1688
Lys Lys Asn Pro Glu Asp Lys Ile Ser Phe Asn Gln Gln Val Thr Ile
375 380 385

tgg tgg gct ggg ctt atg cga ggt gct gtt tct atg gcc ctt gct tat 1736
Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu Ala Tyr
390 395 400

aat cag ttt acc agg gga ggt cat act cag tta cgt gcc aat gca ata 1784
Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn Ala Ile
405 410 415

atg atc acg agt act atc act gtt gtc ctt ttc agc aca gtg gta ttt 1832
Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val Val Phe
420 425 430

ggg ttg atg aca aaa cct tta att cta tta ttg cta ccc tca caa aaa 1880
Gly Leu Met Thr Lys Pro Leu Ile Leu Leu Leu Leu Pro Ser Gln Lys
435 440 445 450

cac ttg atc aga atg atc tcc tct gaa ccg atg act cca aaa tcc ttc 1928
His Leu Ile Arg Met Ile Ser Ser Glu Pro Met Thr Pro Lys Ser Phe
455 460 465

att gtg cca ctt ctt gac agc aca caa gac tca gaa gct gat ctg ggc 1976
Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp Leu Gly
470 475 480

cga cat gta ccc cgt ccc cac agt ttg cgg atg ctc ctg tca acc cca 2024
Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser Thr Pro
485 490 495
tct cac acg gta cat tac tac tgg aga aaa ttt gac aat gca ttc atg 2072
Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala Phe Met
500 505 510
cgt cct gtt ttc ggt gga cga ggt ttt gta cct ttt gtt cca gga tca 2120
Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Val Pro Gly Ser
515 520 525 530
cct act gaa ccg gtc gaa ccg acc gaa cca aga cca gcc gaa tca aga 2168
Pro Thr Glu Pro Val Glu Pro Thr Glu Pro Arg Pro Ala Glu Ser Arg
535 540 545
cca acc gaa cca act gat gag tgattacact gatggagatg caggttgcac 2219
Pro Thr Glu Pro Thr Asp Glu
550
taaagtccca ctggccttgg agaaggacga aggcagtttt ttgggtttga ggttttgttt 2279
actgttaata gttttcgaat gtggttaaaa aagggttgtc tagtttttat atataggtcg 2339
cagatacgta atttcagctc agttcccgag gtgaacccct tagaggtttt cttcctgacg 2399
gtttttcttc tttttgtaa tttatcaaaa acaccaaatg ggtgtatatt ctttaagctt 2459
gtagcttaat taccttataa gcatgtggta gcgttcgtgt aatatgtaaa atttccattg 2519
ccagaaaaga aacttccata caatatttct gccg 2553

<210> 17
<211> 553
<212> PRT
<213> Nierembergia hybrida

<223> Amino acid sequence of protein regulating the pH
of vacuoles

<400> 17
Met Ala Phe Asp Phe Gly Thr Leu Leu Gly Lys Met Asn Asn Leu Thr
5 10 15
Thr Ser Asp His Gln Ser Val Val Ser Val Asn Leu Phe Val Ala Leu
20 25 30

Ile Cys Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp
35 40 45

Met Asn Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Val
50 55 60

Ile Ile Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe
65 70 75 80

Ser Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn
85 90 95

Ala Gly Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr
100 105 110

Ile Met Leu Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile
115 120 125

Ser Ala Gly Ala Ile Gly Ile Phe Lys Lys Met Asp Ile Gly His Leu
130 135 140

Glu Ile Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp
145 150 155 160

Ser Val Cys Thr Leu Gln Val Leu Asn Gln Glu Glu Thr Pro Leu Leu
165 170 175

Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val
180 185 190

Val Leu Phe Asn Ala Val Gln Asn Phe Asp Leu Ser His Ile Ser Thr
195 200 205

Gly Lys Ala Leu Gln Leu Ile Gly Asn Phe Leu Tyr Leu Phe Ala Ser
210 215 220

Ser Thr Phe Leu Gly Val Ala Val Gly Leu Leu Ser Ala Phe Ile Ile
225 230 235 240

Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile
245 250 255

Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr
260 265 270

Leu Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His
275 280 285

Tyr Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His
290 295 300

Thr Phe Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr
305 310 315 320
Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp
325 330 335
Ser Pro Gly Thr Ser Ile Lys Val Ser Ser Ile Leu Leu Gly Leu Val
340 345 350
Leu Val Gly Arg Gly Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn
355 360 365
Leu Thr Lys Lys Asn Pro Glu Asp Lys Ile Ser Phe Asn Gln Gln Val
370 375 380

Thr Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu
385 390 395 400
Ala Tyr Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn
405 410 415
Ala Ile Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val
420 425 430
Val Phe Gly Leu Met Thr Lys Pro Leu Ile Leu Leu Leu Leu Pro Ser
435 440 445
Gln Lys His Leu Ile Arg Met Ile Ser Ser Glu Pro Met Thr Pro Lys
450 455 460
Ser Phe Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp
465 470 475 480
Leu Gly Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser
485 490 495
Thr Pro Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala
500 505 510
Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Val Pro
515 520 525
Gly Ser Pro Thr Glu Pro Val Glu Pro Thr Glu Pro Arg Pro Ala Glu
530 535 540
Ser Arg Pro Thr Glu Pro Thr Asp Glu
545 550

<210> 18
<211> 2361

<212> DNA

<213> Torenia hybrida

<223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles

<400> 18

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gttggagatt ccgagctgca gcatcacctt gcttatgtaa gctttaaaag tatcagaatt    60
gaatatcgac cactggaaag tgttttagga cttggattct tatctattga gcttgtttga   120
aggtgaaaaa aggctcgatc tcgttcctct atagttggtt ttctggagtt gcaagcgact   180
ctactcggaa tctctttccg ccttattgga agctctgctt tactaaaaaa agtttgtctt   240
tttatctctg attcatcata aaatctgcgg gagattcaga agcggagatc tggtgcccag   300
agcaggagtt tcaactttga gcccgtttat atttataaac aaattccgag tccaaagatt   360
gaactttgaa ataatcaaat aatcaagcaa gcaat atg ggg ttt gaa tct gta       413
                                       Met Gly Phe Glu Ser Val
                                                       5
att aag cta gcg gca agt gaa act gac aat ttg tgg agc tct ggt cac      461
Ile Lys Leu Ala Ala Ser Glu Thr Asp Asn Leu Trp Ser Ser Gly His
            10                  15                  20
ggt tca gtg gtc gct ata acc tta ttt gtc act ctt ctc tgc aca tgt      509
Gly Ser Val Val Ala Ile Thr Leu Phe Val Thr Leu Leu Cys Thr Cys
        25                  30                  35
ata gtg att ggt cat ctt ctg gag gaa aac cgt tgg atg aat gaa tct      557
Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn Glu Ser
    40                  45                  50
atc att gcc ctc ata att ggt tta gcc acg gga gtt ata atc ctg tta      605
Ile Ile Ala Leu Ile Ile Gly Leu Ala Thr Gly Val Ile Ile Leu Leu
55                  60                  65                  70
ata agt ggt gga aaa agc tcc cat ctc ttg gtg ttc agt gag gat ctt      653
Ile Ser Gly Gly Lys Ser Ser His Leu Leu Val Phe Ser Glu Asp Leu
                75                  80                  85

ttc ttc atc tat gcg ctg cca cca atc att ttt aat gcg ggg ttc caa      701
Phe Phe Ile Tyr Ala Leu Pro Pro Ile Ile Phe Asn Ala Gly Phe Gln
            90                  95                  100
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gta aaa aag aaa tca ttc ttt cgc aat ttc gca act ata atg atg ttt      749
Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ala Thr Ile Met Met Phe
        105                 110                 115

gga gca gtt ggt acc ttg ata tcc ttc atc atc att tca ctc ggt aca      797
Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile Ser Leu Gly Thr
    120                 125                 130

att gca ttc ttc ccc aaa atg aac atg aga ctt gga gtt gga gat tat      845
Ile Ala Phe Phe Pro Lys Met Asn Met Arg Leu Gly Val Gly Asp Tyr
135                 140                 145                 150

ctt gct att gga gct att ttt gct gca aca gac tca gtt tgc aca tta      893
Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser Val Cys Thr Leu
                155                 160                 165

cag gtg cta agc cag gac gaa aca cca ctg ttg tac agt cta gtg ttt      941
Gln Val Leu Ser Gln Asp Glu Thr Pro Leu Leu Tyr Ser Leu Val Phe
            170                 175                 180

ggc gag ggt gtt gta aat gac gcg act tca gtg gtc cta ttt aat gca      989
Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val Leu Phe Asn Ala
        185                 190                 195

gta cag aac ttc gac ctg cct cat atg tct act gct aaa gct ttc gag     1037
Val Gln Asn Phe Asp Leu Pro His Met Ser Thr Ala Lys Ala Phe Glu
    200                 205                 210

ctt gtt gga aac ttc ttt tat tta ttt gct aca agc act gtg ctg ggt     1085
Leu Val Gly Asn Phe Phe Tyr Leu Phe Ala Thr Ser Thr Val Leu Gly
215                 220                 225                 230

gtt ctg act gga ttg ctt agt gca tac atc ata aaa aag ctc tat ttt     1133
Val Leu Thr Gly Leu Leu Ser Ala Tyr Ile Ile Lys Lys Leu Tyr Phe
                235                 240                 245

gga agg cac tcc act gat cgc gag gtt gcc ata atg ata ctc atg gct     1181
Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile Leu Met Ala
            250                 255                 260

tat ctg tcg tat atg tta gct gaa tta ttc gat ttg agc ggt atc ctc     1229
Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Asp Leu Ser Gly Ile Leu
        265                 270                 275

acc gtg ttc ttc tgt gga att gtg atg tcg cac tat aca tgg cac aat     1277
Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr Thr Trp His Asn
    280                 285                 290
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gtc act gaa aac tca aga gtt acc acc aag cat aca ttt gcg aca ttg 1325
Val Thr Glu Asn Ser Arg Val Thr Thr Lys His Thr Phe Ala Thr Leu
295 300 305 310

tca ttt gtt gct gaa ata ttt ata ttt ctg tat gtt ggc atg gat gct 1373
Ser Phe Val Ala Glu Ile Phe Ile Phe Leu Tyr Val Gly Met Asp Ala
315 320 325

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<223> Amino acid sequence of protein regulating the pH of vacuoles

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35 40 45

Arg Trp Met Asn Glu Ser Ile Ile Ala Leu Ile Ile Gly Leu Ala Thr
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Gly Val Ile Ile Leu Leu Ile Ser Gly Gly Lys Ser Ser His Leu Leu
65 70 75 80

Val Phe Ser Glu Asp Leu Phe Phe Ile Tyr Ala Leu Pro Pro Ile Ile
85 90 95

Phe Asn Ala Gly Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe
100 105 110

Ala Thr Ile Met Met Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile
115 120 125

Ile Ile Ser Leu Gly Thr Ile Ala Phe Phe Pro Lys Met Asn Met Arg
130 135 140

Leu Gly Val Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr
145 150 155 160

Asp Ser Val Cys Thr Leu Gln Val Leu Ser Gln Asp Glu Thr Pro Leu
165 170 175

Leu Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser
180 185 190

Val Val Leu Phe Asn Ala Val Gln Asn Phe Asp Leu Pro His Met Ser
195 200 205

Thr Ala Lys Ala Phe Glu Leu Val Gly Asn Phe Phe Tyr Leu Phe Ala
210 215 220

Thr Ser Thr Val Leu Gly Val Leu Thr Gly Leu Leu Ser Ala Tyr Ile
225 230 235 240

Ile Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala
245 250 255

Ile Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe
260 265 270

Asp Leu Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser
275 280 285

His Tyr Thr Trp His Asn Val Thr Glu Asn Ser Arg Val Thr Thr Lys
290 295 300

His Thr Phe Ala Thr Leu Ser Phe Val Ala Glu Ile Phe Ile Phe Leu
305 310 315 320

Tyr Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Arg Phe Val Ser
325 330 335

Gly Ser Met Thr Thr Ser Ala Ala Val Ser Ala Thr Leu Leu Gly Leu
340 345 350

Val Leu Leu Ser Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser
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Asn Leu Ala Lys Lys Ser Pro Leu Glu Lys Ile Ser Leu Arg Gln Gln
370 375 380

Ile Ile Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala
385 390 395 400

Leu Ala Tyr Lys Gln Phe Thr Arg Glu Gly Leu Thr Val Glu Arg Glu
405 410 415

Asn Ala Ile Phe Ile Thr Ser Thr Ile Thr Ile Val Leu Phe Ser Thr
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Val Val Phe Gly Leu Met Thr Lys Pro Leu Ile Asn Leu Leu Ile Pro
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<110> Iida, Shigeru
Tanaka, Sachiko
Inagaki, Yoshishige

<120> Genes Encoding Proteins Regulating the pH of Vacuoles

<130> 001560-397

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Cys Ala Cys Ile Val Leu Gly His Leu Leu Glu Glu Asn Arg Trp Val
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Asn Glu Ser Ile Thr Ala Leu Ile Ile Gly Leu Cys Thr Gly Val Val
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Ile Leu Leu Leu Ser Gly Gly Lys Ser Ser His Leu Leu Val Phe Ser
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agt ccg aag cac ttc acg gtg cca ctc ctg gac aac caa cct gac tca    1739
Ser Pro Lys His Phe Thr Val Pro Leu Leu Asp Asn Gln Pro Asp Ser
465                 470                 475                 480

gaa agc gat atg ata acc gga cct gag gtt gct cga cca act gcc ttg    1787
Glu Ser Asp Met Ile Thr Gly Pro Glu Val Ala Arg Pro Thr Ala Leu
                485                 490                 495

cgc atg ctg cta agg acg cca acc cac acc gtg cac cgc tac tgg cgt    1835
Arg Met Leu Leu Arg Thr Pro Thr His Thr Val His Arg Tyr Trp Arg
```

500 505 510

aag ttt gat gat tcg ttt atg cgt ccc gtg ttt ggc ggg cgg gga ttc 1883
Lys Phe Asp Asp Ser Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe
515 520 525

gtt ccg ttt gtc gcg ggc tca cca gtt gag cag agc cct aga tga 1928
Val Pro Phe Val Ala Gly Ser Pro Val Glu Gln Ser Pro Arg
530 535 540

ggtacaaagt acaaacaaga cactgttgct gggtgaaata gtgtaagttg tatcatagtt 1988

gattctggtt gcccctctta tgaaatgggc tgggtgaaag tcttctcact agctaggttg 2048

cattgcattg ctacttcata aatgttttat tttattttgt aaatgttggt gcattttagg 2108

tacttgtatt aacacctcat ttgtagcata ttatttggta cagagtattt tttttatgaa 2168

acaataatgg ctgaattatc aatttggctc tatgttttga tgcttagtaa aaaaaaaaaa 2228

aaaaaaaaa 2237

<210> 2
<211> 542
<212> PRT
<213> Ipomea nil

<220>
<221> peptide
<222> (1)..(542)
<223> Amino acid sequence of protein regulating the pH of vacuoles

<400> 2

Met Ala Phe Gly Leu Ser Ser Leu Leu Gln Asn Ser Asp Leu Phe Thr
1 5 10 15

Ser Asp His Ala Ser Val Val Ser Met Asn Leu Phe Val Ala Leu Leu
20 25 30

Cys Ala Cys Ile Val Leu Gly His Leu Leu Glu Glu Asn Arg Trp Val
35 40 45

Asn Glu Ser Ile Thr Ala Leu Ile Ile Gly Leu Cys Thr Gly Val Val
50 55 60

Ile Leu Leu Leu Ser Gly Gly Lys Ser Ser His Leu Leu Val Phe Ser
65 70 75 80

Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala
85 90 95

Gly Phe Gln Val Lys Lys Lys Gln Phe Phe Val Asn Phe Met Thr Ile
100 105 110

Met Leu Phe Gly Ala Ile Gly Thr Leu Ile Ser Cys Ser Ile Ile Ser
115 120 125

Phe Gly Ala Val Lys Ile Phe Lys His Leu Asp Ile Asp Phe Leu Asp
130 135 140

Phe Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser
145 150 155 160

Val Cys Thr Leu Gln Val Leu Ser Gln Asp Glu Thr Pro Leu Leu Tyr
165 170 175

Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val
180 185 190

Leu Phe Asn Ala Ile Gln Ser Phe Asp Met Thr Ser Phe Asp Pro Lys
195 200 205

Ile Gly Leu His Phe Ile Gly Asn Phe Leu Tyr Leu Phe Leu Ser Ser
210 215 220

Thr Phe Leu Gly Val Gly Ile Gly Leu Leu Cys Ala Tyr Ile Ile Lys
225 230 235 240

Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Leu Met
245 250 255

Met Leu Met Ser Tyr Leu Ser Tyr Ile Met Ala Glu Leu Phe Tyr Leu
260 265 270

Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr
275 280 285

Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Arg His Ser
290 295 300

Phe Ala Thr Leu Ser Phe Val Ala Glu Thr Phe Ile Phe Leu Tyr Val
305 310 315 320

Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Lys Asn Ser
325 330 335

Gln Gly Leu Ser Val Ala Val Ser Ser Ile Leu Val Gly Leu Ile Leu
340 345 350

Val Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu
355 360 365

Ala Lys Lys Asn Ser Ser Asp Lys Ile Ser Phe Arg Gln Gln Ile Ile
370 375 380

Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Ile Ala Leu Ala
385 390 395 400

Tyr Asn Lys Phe Thr Thr Ser Gly His Thr Ser Leu His Glu Asn Ala
405 410 415

Ile Met Ile Thr Ser Thr Val Thr Val Val Leu Phe Ser Thr Val Val
420 425 430

Phe Gly Leu Met Thr Lys Pro Leu Ile Asn Leu Leu Leu Pro Pro His
435 440 445

Lys Gln Met Pro Ser Gly His Ser Ser Met Thr Thr Ser Glu Pro Ser
450 455 460

Ser Pro Lys His Phe Thr Val Pro Leu Leu Asp Asn Gln Pro Asp Ser
465 470 475 480

Glu Ser Asp Met Ile Thr Gly Pro Glu Val Ala Arg Pro Thr Ala Leu
485 490 495

Arg Met Leu Leu Arg Thr Pro Thr His Thr Val His Arg Tyr Trp Arg
500 505 510

Lys Phe Asp Asp Ser Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe
515 520 525

Val Pro Phe Val Ala Gly Ser Pro Val Glu Gln Ser Pro Arg
530 535 540

<210> 3
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> MseI adaptor

<400> 3
gacgatgagt cctgag 16

<210> 4
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> MseI adaptor

<400> 4
tactcaggac tcat 14

<210> 5
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> TIR primer

<400> 5
tgtgcatttt tcttgtagtg 20

<210> 6
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> MseI primer

<400> 6
gatgagtcct gagtaa 16

<210> 7
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> TIR+N primer

<220>
<221> misc_feature
<222> (19)..(19)
<223> Nucleotide 19 = "n" wherein "n" = any nucleotide

<400> 7
tgtgcatttt tcttgtagn 19

<210> 8
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> MseI+N primer

<220>
<221> misc_feature
<222> (17)..(17)
<223> Nucleotide 17 = "n" wherein "n" = any nucleotide

<400> 8
gatgagtcct gagtaan 17

<210> 9
<211> 130
<212> DNA
<213> Artificial Sequence

<220>

<223> MseI+N primer

<400> 9
tgagcatttt tcttgtagtg ctgagatttt cctccatttg tctgaagctc ttcatccttc 60

aacactaccc ccacatctca cctttcaagg tccaatcttt atcattcatc tttactcagg 120

actcatcgtc 130

<210> 10
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> PR-5 primer

<400> 10
gggatccaac aaaaatggct gtcggg 26

<210> 11
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> PR-3 primer

<400> 11
gggtcgacta agcatcaaaa catagagcc 29

<210> 12
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> CBSC1-linker

<400> 12
cgatagatct gggggtcgac at 22

<210> 13
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> CBSC2-linker

<400> 13
cgatgtcgac ccccagatct at 22

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<210>  14
<211>  2423
<212>  DNA
<213>  Petunia hybrida

<220>
<221>  misc_feature
<222>  (1)..(2423)
<223>  Nucleotide sequence of DNA encoding for protein regulating the
       pH of vacuoles

<400> 14
attgcgcttc gtattttact gctgaatgaa atcgtgtttt tttattcagt tcgttgttat    60

taatttcaga gtttttttta ttaaaggtgt gtttggttga agaaattgta tttgctgaat   120

tttgcagaag tttttgagtt tttgctaaac tattgtgaga tctgattttg aatttttcca   180

gtggtgtttt aagctcaatt cgacgtcgtt tttactggaa ttctgatcag taaatagggc   240

tattttgatg taaggttgtg aaagtttaca gtttggaagt tgagttagtg aaaaagggga   300

aactttattg tgatattttc acaagtattt ggtgaattca ggttattgag a atg gct    357
                                                         Met Ala

ttt gat ttt ggg acg ttg ttg gga aat gta gac agg tta tcg aca tct     405
Phe Asp Phe Gly Thr Leu Leu Gly Asn Val Asp Arg Leu Ser Thr Ser
        5                   10                  15

gat cat caa tca gtt gtg tcg ata aac tta ttc gtt gct ctt att tgc     453
Asp His Gln Ser Val Val Ser Ile Asn Leu Phe Val Ala Leu Ile Cys
    20                  25                  30

gcg tgt att gtg atc ggt cat ttg ttg gaa gaa aac aga tgg atg aat     501
Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn
35                  40                  45                  50

gag tcc ata act gcc tta gtg att ggt tct tgt act gga atc gtt att     549
Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Ile Val Ile
                55                  60                  65

cta ctg ata agt gga gga aag aac tct cat att tta gtg ttc agt gaa     597
Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe Ser Glu
            70                  75                  80

gat ctt ttc ttc att tac ctt ctt ccg cca atc att ttt aat gct ggg     645
Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala Gly
        85                  90                  95

ttc cag gtg aaa aag aaa tcg ttc ttc cgc aat ttc agc act atc atg     693
Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr Ile Met
    100                 105                 110

ctc ttt ggg gca ctt ggc acc ttg ata tca ttc att att ata tca tta     741
Leu Phe Gly Ala Leu Gly Thr Leu Ile Ser Phe Ile Ile Ile Ser Leu
115                 120                 125                 130
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ggt gcc att ggc att ttc aag aaa atg aat att gga agc ctt gaa att      789
Gly Ala Ile Gly Ile Phe Lys Lys Met Asn Ile Gly Ser Leu Glu Ile
                135                 140                 145

gga gat tac ctt gca att ggg gca atc ttc tct gct aca gat tct gta      837
Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ser Ala Thr Asp Ser Val
            150                 155                 160

tgc acc tta caa gtg ctt aat cag gat gaa aca ccc tta ttg tac agt      885
Cys Thr Leu Gln Val Leu Asn Gln Asp Glu Thr Pro Leu Leu Tyr Ser
        165                 170                 175

cta gtt ttt ggg gaa ggt gtt gtg aat gat gcc aca tct gta gtt ctg      933
Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val Leu
    180                 185                 190

ttc aat gct atc cag aac ttt gac tta tct cac atc gac acg ggc aaa      981
Phe Asn Ala Ile Gln Asn Phe Asp Leu Ser His Ile Asp Thr Gly Lys
195                 200                 205                 210

gct atg gaa tta gtt gga aac ttt cta tac ttg ttt gcc tca agc act     1029
Ala Met Glu Leu Val Gly Asn Phe Leu Tyr Leu Phe Ala Ser Ser Thr
                215                 220                 225

gcc cta gga gtt gct gct ggc cta ctg agc gcc tat att att aaa aaa     1077
Ala Leu Gly Val Ala Ala Gly Leu Leu Ser Ala Tyr Ile Ile Lys Lys
            230                 235                 240

ctc tac ttt gga agg cac tca act gac cgt gag gtt gct ata atg ata     1125
Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile
        245                 250                 255

ctc atg gct tac cta tct tac atg ctt gct gaa tta ttc tat tta agt     1173
Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr Leu Ser
    260                 265                 270

gca atc ctc act gtg ttt ttc tct ggg atc gtg atg tct cac tac acc     1221
Ala Ile Leu Thr Val Phe Phe Ser Gly Ile Val Met Ser His Tyr Thr
275                 280                 285                 290

tgg cat aat gtg act gag agc tcg aga gtc act acc aag cac act ttt     1269
Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His Thr Phe
                295                 300                 305

gct aca tta tca ttt att gct gaa ata ttc ata ttc ctt tat gtt ggt     1317
Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr Val Gly
            310                 315                 320

atg gat gct ttg gac att gag aag tgg aag ttt gta agc gac agc cct     1365
Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp Ser Pro
        325                 330                 335

gga ata tca gtt cag gtt agc tca ata ttg ctg ggt ctt gtt ttg gtt     1413
Gly Ile Ser Val Gln Val Ser Ser Ile Leu Leu Gly Leu Val Leu Val
    340                 345                 350

gga aga gca gca ttt gtt ttc cca ttg tca ttc ttg tcc aac ttg acc     1461
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Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu Thr
355 360 365 370

aag aaa act cca gag gcg aaa att agt ttt aac cag cag gtt aca ata 1509
Lys Lys Thr Pro Glu Ala Lys Ile Ser Phe Asn Gln Gln Val Thr Ile
375 380 385

tgg tgg gct gga ctt atg aga ggt gcc gtt tct atg gcc ctt gct tat 1557
Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu Ala Tyr
390 395 400

aat cag ttt acc agg gga ggt cat act cag tta cgc gca aat gca ata 1605
Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn Ala Ile
405 410 415

atg atc aca agt act atc act gtt gtc ctt ttc agc aca gtc gtg ttt 1653
Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val Val Phe
420 425 430

ggg ttg atg aca aaa cct ttg att aga ata ttg cta ccc tca cac aaa 1701
Gly Leu Met Thr Lys Pro Leu Ile Arg Ile Leu Leu Pro Ser His Lys
435 440 445 450

cac ttg agc aga atg atc tct tct gaa cca acg acc cca aaa tcc ttc 1749
His Leu Ser Arg Met Ile Ser Ser Glu Pro Thr Thr Pro Lys Ser Phe
455 460 465

att gtg cca ctt ctt gac agc aca caa gac tca gaa gct gat ctg gaa 1797
Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp Leu Glu
470 475 480

cgc cat gta ccc cgt ccc cac agt ttg cgg atg ctc ctt tca acc cca 1845
Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser Thr Pro
485 490 495

tct cat aca gtg cat tat tac tgg aga aag ttt gac aat gca ttc atg 1893
Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala Phe Met
500 505 510

cgt cca gtt ttc ggt gga cga ggt ttt gta cct ttt gct cca gga tca 1941
Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Ala Pro Gly Ser
515 520 525 530

ccg aca gac cca gtt ggt gga aat ttg caa tgatggagat acagattgca 1991
Pro Thr Asp Pro Val Gly Gly Asn Leu Gln
535 540

aaaagtggtc ttggtgaggg aagagggcag tttttggta atgaggttcc gttttcttta 2051

atgttaatag caagtgtggt taaaaagggg ttgtctagtt tataggtttt gcagatctca 2111

agtatattca tttgggtgat catgttttca gctcagttat tgcttttggt cattgctgac 2171

catcaatttc tgtggggaat tcctataggt tttctcccta acagttcttt tcttcatctt 2231

tttgcaattt atcgaaacac caaatgggtg tatattctgt aagcttgtgg catagctagc 2291

ttaattgtct tgtaaaattt cctacaggtt agagattggt tcttgatatg tagatttcat 2351

atgattgtaa cattcccatt tctcagaaaa gaaactataa tataaaattt ctggtggctg 2411

tcgcccgtgc tc 2423

<210> 15
<211> 540
<212> PRT
<213> Petunia hybrida

<220>
<221> peptide
<222> (1)..(540)
<223> Amino acid sequence of protein regulating the pH of vacuoles

<400> 15

Met Ala Phe Asp Phe Gly Thr Leu Leu Gly Asn Val Asp Arg Leu Ser
1 5 10 15

Thr Ser Asp His Gln Ser Val Val Ser Ile Asn Leu Phe Val Ala Leu
20 25 30

Ile Cys Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp
35 40 45

Met Asn Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Ile
50 55 60

Val Ile Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe
65 70 75 80

Ser Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn
85 90 95

Ala Gly Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr
100 105 110

Ile Met Leu Phe Gly Ala Leu Gly Thr Leu Ile Ser Phe Ile Ile Ile
115 120 125

Ser Leu Gly Ala Ile Gly Ile Phe Lys Lys Met Asn Ile Gly Ser Leu
130 135 140

Glu Ile Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ser Ala Thr Asp
145 150 155 160

Ser Val Cys Thr Leu Gln Val Leu Asn Gln Asp Glu Thr Pro Leu Leu
165 170 175

Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val
180 185 190

Val Leu Phe Asn Ala Ile Gln Asn Phe Asp Leu Ser His Ile Asp Thr
195 200 205

Gly Lys Ala Met Glu Leu Val Gly Asn Phe Leu Tyr Leu Phe Ala Ser
210 215 220

Ser Thr Ala Leu Gly Val Ala Ala Gly Leu Leu Ser Ala Tyr Ile Ile
225 230 235 240

Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile
245 250 255

Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr
260 265 270

Leu Ser Ala Ile Leu Thr Val Phe Phe Ser Gly Ile Val Met Ser His
275 280 285

Tyr Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His
290 295 300

Thr Phe Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr
305 310 315 320

Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp
325 330 335

Ser Pro Gly Ile Ser Val Gln Val Ser Ser Ile Leu Leu Gly Leu Val
340 345 350

Leu Val Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn
355 360 365

Leu Thr Lys Lys Thr Pro Glu Ala Lys Ile Ser Phe Asn Gln Gln Val
370 375 380

Thr Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu
385 390 395 400

Ala Tyr Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn
405 410 415

Ala Ile Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val
420 425 430

Val Phe Gly Leu Met Thr Lys Pro Leu Ile Arg Ile Leu Leu Pro Ser
435 440 445

His Lys His Leu Ser Arg Met Ile Ser Ser Glu Pro Thr Thr Pro Lys
450 455 460

Ser Phe Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp
465 470 475 480

Leu Glu Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser
485 490 495

Thr Pro Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala
500 505 510

Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Ala Pro
515 520 525

Gly Ser Pro Thr Asp Pro Val Gly Gly Asn Leu Gln
530 535 540

<210> 16
<211> 2553
<212> DNA
<213> Nierembergia hybrida

<220>
<221> misc_feature
<222> (1)..(2553)
<223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles

<400> 16
aattattatt atttctctcc aactctcatt tctcagtttg ttgtgacttt ttcagagctt 60

gaagttcagt taattcattt tccaatatat tgattgtttt catttgagcg cgagaggatt 120

tcgtcttctc aatctgcttt caaatccttt ttgtttgtga tattcgatat tattcactca 180

gtttacctta atatttcctc gcactttctg aattcgagtg ctttgaagtg tgttggattt 240

cgaaaagcgg aagaaaattc agcaaaaacg ctgttgctga atttgcagca gtttgagttt 300

ttgctaaata gctaagatct gattgaattt ttcactggtg cttataggga aattcgacgt 360

cgttttgact gcaatatttg tccgtgattc ggactttgtt gaaattttgc tatttgaaat 420

ttgaatgtaa ggttgtcata gctttgccac tcggaaatac agtcagtgag aaagaaaaaa 480

aactgtgtag tgtttttttcc acaagtattt ggtgaattga ggttcttgaa atg gcg 536
Met Ala

ttt gac ttt ggg act ctg ctg gga aag atg aac aac tta aca act tct 584
Phe Asp Phe Gly Thr Leu Leu Gly Lys Met Asn Asn Leu Thr Thr Ser
5 10 15

gat cat caa tca gtg gtg tcg gta aac ttg ttt gtt gca ctt att tgc 632
Asp His Gln Ser Val Val Ser Val Asn Leu Phe Val Ala Leu Ile Cys
20 25 30

gcg tgt att gtg atc ggt cat tta ttg gag gaa aac aga tgg atg aat 680
Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn
35 40 45 50

gag tcc ata act gcc ctt gtg att ggt agt tgc act gga gtc atc att 728
Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Val Ile Ile
55 60 65

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cta cta ata agt gga gga aag aac tca cat att tta gtg ttc agc gaa      776
Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe Ser Glu
                70                  75                  80

gat ctt ttc ttc att tac ctt ctt cca ccg atc att ttt aat gct ggg      824
Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala Gly
            85                  90                  95

ttc cag gtg aaa aag aaa tca ttc ttc cgc aat ttc agt act atc atg      872
Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr Ile Met
        100                 105                 110

ctc ttt ggg gca gtt ggc acc ttg ata tcg ttc att att ata tca gcg      920
Leu Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile Ser Ala
115                 120                 125                 130

ggt gct att ggc att ttc aag aaa atg gat att gga cac ctt gaa att      968
Gly Ala Ile Gly Ile Phe Lys Lys Met Asp Ile Gly His Leu Glu Ile
                135                 140                 145

gga gat tac ctt gca att gga gca atc ttt gct gca aca gat tct gta     1016
Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser Val
                150                 155                 160

tgc acc tta caa gtg ctt aat cag gaa gaa aca ccg tta ttg tac agt     1064
Cys Thr Leu Gln Val Leu Asn Gln Glu Glu Thr Pro Leu Leu Tyr Ser
            165                 170                 175

cta gtg ttt gga gaa ggt gtt gtg aat gat gcc aca tct gta gtg ctg     1112
Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val Leu
        180                 185                 190

ttc aat gct gtc cag aac ttt gac tta tct cat atc agc aca ggc aaa     1160
Phe Asn Ala Val Gln Asn Phe Asp Leu Ser His Ile Ser Thr Gly Lys
195                 200                 205                 210

gct ctg caa tta att gga aac ttt cta tac ttg ttt gcc tcg agc act     1208
Ala Leu Gln Leu Ile Gly Asn Phe Leu Tyr Leu Phe Ala Ser Ser Thr
                215                 220                 225

ttc cta ggg gtt gct gtt ggc cta cta agt gcc ttt ata att aag aaa     1256
Phe Leu Gly Val Ala Val Gly Leu Leu Ser Ala Phe Ile Ile Lys Lys
                230                 235                 240

ctc tac ttt gga agg cac tcg act gat cgt gag gtt gct ata atg ata     1304
Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile
            245                 250                 255

ctc atg gcg tac cta tca tac atg ctt gct gaa tta ttc tat tta agt     1352
Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr Leu Ser
        260                 265                 270

gga atc ctc act gtg ttt ttc tgt ggg atc gtg atg tct cac tat acc     1400
Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr Thr
275                 280                 285                 290

tgg cat aat gtg act gag agc tca aga gtc act acc aag cac acg ttt     1448
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Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His Thr Phe
                295                 300                 305

gct aca tta tca ttt att gct gaa ata ttc ata ttc ctt tat gtt ggt     1496
Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr Val Gly
            310                 315                 320

atg gat gct ttg gac att gag aag tgg aag ttt gta agc gac agc ccc     1544
Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp Ser Pro
        325                 330                 335

gga aca tca att aag gtc agc tca att ctg cta ggt ctt gtt ttg gtt     1592
Gly Thr Ser Ile Lys Val Ser Ser Ile Leu Leu Gly Leu Val Leu Val
    340                 345                 350

gga agg gga gcc ttt gtt ttc ccc ttg tca ttc ttg tcc aac ttg acc     1640
Gly Arg Gly Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu Thr
355                 360                 365                 370

aag aaa aat cct gag gac aag att agc ttt aac cag cag gtt aca ata     1688
Lys Lys Asn Pro Glu Asp Lys Ile Ser Phe Asn Gln Gln Val Thr Ile
                375                 380                 385

tgg tgg gct ggg ctt atg cga ggt gct gtt tct atg gcc ctt gct tat     1736
Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu Ala Tyr
            390                 395                 400

aat cag ttt acc agg gga ggt cat act cag tta cgt gcc aat gca ata     1784
Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn Ala Ile
        405                 410                 415

atg atc acg agt act atc act gtt gtc ctt ttc agc aca gtg gta ttt     1832
Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val Val Phe
    420                 425                 430

ggg ttg atg aca aaa cct tta att cta tta ttg cta ccc tca caa aaa     1880
Gly Leu Met Thr Lys Pro Leu Ile Leu Leu Leu Leu Pro Ser Gln Lys
435                 440                 445                 450

cac ttg atc aga atg atc tcc tct gaa ccg atg act cca aaa tcc ttc     1928
His Leu Ile Arg Met Ile Ser Ser Glu Pro Met Thr Pro Lys Ser Phe
                455                 460                 465

att gtg cca ctt ctt gac agc aca caa gac tca gaa gct gat ctg ggc     1976
Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp Leu Gly
            470                 475                 480

cga cat gta ccc cgt ccc cac agt ttg cgg atg ctc ctg tca acc cca     2024
Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser Thr Pro
        485                 490                 495

tct cac acg gta cat tac tac tgg aga aaa ttt gac aat gca ttc atg     2072
Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala Phe Met
    500                 505                 510

cgt cct gtt ttc ggt gga cga ggt ttt gta cct ttt gtt cca gga tca     2120
Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Val Pro Gly Ser
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515             520                        525                   530

cct act gaa ccg gtc gaa ccg acc gaa cca aga cca gcc gaa tca aga    2168
Pro Thr Glu Pro Val Glu Pro Thr Glu Pro Arg Pro Ala Glu Ser Arg
                535                 540                 545

cca acc gaa cca act gat gag tgattacact gatggagatg caggttgcac      2219
Pro Thr Glu Pro Thr Asp Glu
            550

taaagtccca ctggccttgg agaaggacga aggcagtttt ttgggtttga ggttttgttt 2279

actgttaata gttttcgaat gtggttaaaa aagggttgtc tagtttttat atataggtcg 2339

cagatacgta atttcagctc agttcccgag gtgaacccct tagaggtttt cttcctgacg 2399

gtttttcttc tttttgtaa tttatcaaaa acaccaaatg ggtgtatatt ctttaagctt 2459

gtagcttaat taccttataa gcatgtggta gcgttcgtgt aatatgtaaa atttccattg 2519

ccagaaaaga aacttccata caatatttct gccg                             2553


<210>  17
<211>  553
<212>  PRT
<213>  Nierembergia hybrida

<220>
<221>  peptide
<222>  (1)..(553)
<223>  Amino acid sequence of protein regulating the pH of vacuoles

<400> 17

Met Ala Phe Asp Phe Gly Thr Leu Leu Gly Lys Met Asn Asn Leu Thr
1               5                   10                  15

Thr Ser Asp His Gln Ser Val Val Ser Val Asn Leu Phe Val Ala Leu
            20                  25                  30

Ile Cys Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp
        35                  40                  45

Met Asn Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Val
    50                  55                  60

Ile Ile Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe
65                  70                  75                  80

Ser Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn
                85                  90                  95

Ala Gly Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr
            100                 105                 110

Ile Met Leu Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile
```

115 120 125

Ser Ala Gly Ala Ile Gly Ile Phe Lys Lys Met Asp Ile Gly His Leu
130 135 140

Glu Ile Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp
145 150 155 160

Ser Val Cys Thr Leu Gln Val Leu Asn Gln Glu Glu Thr Pro Leu Leu
165 170 175

Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val
180 185 190

Val Leu Phe Asn Ala Val Gln Asn Phe Asp Leu Ser His Ile Ser Thr
195 200 205

Gly Lys Ala Leu Gln Leu Ile Gly Asn Phe Leu Tyr Leu Phe Ala Ser
210 215 220

Ser Thr Phe Leu Gly Val Ala Val Gly Leu Leu Ser Ala Phe Ile Ile
225 230 235 240

Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile
245 250 255

Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr
260 265 270

Leu Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His
275 280 285

Tyr Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His
290 295 300

Thr Phe Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr
305 310 315 320

Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp
325 330 335

Ser Pro Gly Thr Ser Ile Lys Val Ser Ser Ile Leu Leu Gly Leu Val
340 345 350

Leu Val Gly Arg Gly Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn
355 360 365

Leu Thr Lys Lys Asn Pro Glu Asp Lys Ile Ser Phe Asn Gln Gln Val
370 375 380

Thr Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu
385 390 395 400

Ala Tyr Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn
405 410 415

Ala Ile Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val
420 425 430

Val Phe Gly Leu Met Thr Lys Pro Leu Ile Leu Leu Leu Leu Pro Ser
435 440 445

Gln Lys His Leu Ile Arg Met Ile Ser Ser Glu Pro Met Thr Pro Lys
450 455 460

Ser Phe Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp
465 470 475 480

Leu Gly Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser
485 490 495

Thr Pro Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala
500 505 510

Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Val Pro
515 520 525

Gly Ser Pro Thr Glu Pro Val Glu Pro Thr Glu Pro Arg Pro Ala Glu
530 535 540

Ser Arg Pro Thr Glu Pro Thr Asp Glu
545 550

<210> 18
<211> 2361
<212> DNA
<213> Torenia hybrida

<220>
<221> misc_feature
<222> (1)..(2361)
<223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles

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gaatatcgac cactggaaag tgttttagga cttggattct tatctattga gcttgtttga 120

aggtgaaaaa aggctcgatc tcgttcctct atagttggtt ttctggagtt gcaagcgact 180

ctactcggaa tctctttccg ccttattgga agctctgctt tactaaaaaa agtttgtctt 240

tttatctctg attcatcata aaatctgcgg gagattcaga agcggagatc tggtgcccag 300

agcaggagtt tcaactttga gcccgtttat atttataaac aaattccgag tccaaagatt 360

gaactttgaa ataatcaaat aatcaagcaa gcaat atg ggg ttt gaa tct gta 413
Met Gly Phe Glu Ser Val
5

att aag cta gcg gca agt gaa act gac aat ttg tgg agc tct ggt cac 461

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Ile Lys Leu Ala Ala Ser Glu Thr Asp Asn Leu Trp Ser Ser Gly His
            10                  15                  20

ggt tca gtg gtc gct ata acc tta ttt gtc act ctt ctc tgc aca tgt      509
Gly Ser Val Val Ala Ile Thr Leu Phe Val Thr Leu Leu Cys Thr Cys
        25                  30                  35

ata gtg att ggt cat ctt ctg gag gaa aac cgt tgg atg aat gaa tct      557
Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn Glu Ser
    40                  45                  50

atc att gcc ctc ata att ggt tta gcc acg gga gtt ata atc ctg tta      605
Ile Ile Ala Leu Ile Ile Gly Leu Ala Thr Gly Val Ile Ile Leu Leu
55                  60                  65                  70

ata agt ggt gga aaa agc tcc cat ctc ttg gtg ttc agt gag gat ctt      653
Ile Ser Gly Gly Lys Ser Ser His Leu Leu Val Phe Ser Glu Asp Leu
                75                  80                  85

ttc ttc atc tat gcg ctg cca cca atc att ttt aat gcg ggg ttc caa      701
Phe Phe Ile Tyr Ala Leu Pro Pro Ile Ile Phe Asn Ala Gly Phe Gln
            90                  95                  100

gta aaa aag aaa tca ttc ttt cgc aat ttc gca act ata atg atg ttt      749
Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ala Thr Ile Met Met Phe
        105                 110                 115

gga gca gtt ggt acc ttg ata tcc ttc atc atc att tca ctc ggt aca      797
Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile Ser Leu Gly Thr
    120                 125                 130

att gca ttc ttc ccc aaa atg aac atg aga ctt gga gtt gga gat tat      845
Ile Ala Phe Phe Pro Lys Met Asn Met Arg Leu Gly Val Gly Asp Tyr
135                 140                 145                 150

ctt gct att gga gct att ttt gct gca aca gac tca gtt tgc aca tta      893
Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser Val Cys Thr Leu
                155                 160                 165

cag gtg cta agc cag gac gaa aca cca ctg ttg tac agt cta gtg ttt      941
Gln Val Leu Ser Gln Asp Glu Thr Pro Leu Leu Tyr Ser Leu Val Phe
            170                 175                 180

ggc gag ggt gtt gta aat gac gcg act tca gtg gtc cta ttt aat gca      989
Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val Leu Phe Asn Ala
        185                 190                 195

gta cag aac ttc gac ctg cct cat atg tct act gct aaa gct ttc gag     1037
Val Gln Asn Phe Asp Leu Pro His Met Ser Thr Ala Lys Ala Phe Glu
    200                 205                 210

ctt gtt gga aac ttc ttt tat tta ttt gct aca agc act gtg ctg ggt     1085
Leu Val Gly Asn Phe Phe Tyr Leu Phe Ala Thr Ser Thr Val Leu Gly
215                 220                 225                 230

gtt ctg act gga ttg ctt agt gca tac atc ata aaa aag ctc tat ttt     1133
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Val Leu Thr Gly Leu Leu Ser Ala Tyr Ile Ile Lys Lys Leu Tyr Phe
235 240 245

gga agg cac tcc act gat cgc gag gtt gcc ata atg ata ctc atg gct 1181
Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile Leu Met Ala
250 255 260

tat ctg tcg tat atg tta gct gaa tta ttc gat ttg agc ggt atc ctc 1229
Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Asp Leu Ser Gly Ile Leu
265 270 275

acc gtg ttc ttc tgt gga att gtg atg tcg cac tat aca tgg cac aat 1277
Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr Thr Trp His Asn
280 285 290

gtc act gaa aac tca aga gtt acc acc aag cat aca ttt gcg aca ttg 1325
Val Thr Glu Asn Ser Arg Val Thr Thr Lys His Thr Phe Ala Thr Leu
295 300 305 310

tca ttt gtt gct gaa ata ttt ata ttt ctg tat gtt ggc atg gat gct 1373
Ser Phe Val Ala Glu Ile Phe Ile Phe Leu Tyr Val Gly Met Asp Ala
315 320 325

tta gac att gag aaa tgg aga ttc gta agc ggc agc atg aca aca tct 1421
Leu Asp Ile Glu Lys Trp Arg Phe Val Ser Gly Ser Met Thr Thr Ser
330 335 340

gca gct gtc agt gca act ctg ctg gga ttg gtt ttg ctc tca aga gca 1469
Ala Ala Val Ser Ala Thr Leu Leu Gly Leu Val Leu Leu Ser Arg Ala
345 350 355

gcc ttt gta ttc cct tta tca ttt ctc tcc aat ctg gcc aaa aag tcc 1517
Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu Ala Lys Lys Ser
360 365 370

cca ctc gaa aaa atc agt ctc agg cag caa att ata ata tgg tgg gct 1565
Pro Leu Glu Lys Ile Ser Leu Arg Gln Gln Ile Ile Ile Trp Trp Ala
375 380 385 390

ggt ctt atg cgc gga gcc gtt tcc atg gct ctt gct tac aag cag ttt 1613
Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu Ala Tyr Lys Gln Phe
395 400 405

act aga gaa ggt ctc aca gtg gaa cgt gaa aat gcc ata ttc atc acc 1661
Thr Arg Glu Gly Leu Thr Val Glu Arg Glu Asn Ala Ile Phe Ile Thr
410 415 420

agt aca atc acc att gtg ctc ttc agc act gtg gtg ttt ggt ttg atg 1709
Ser Thr Ile Thr Ile Val Leu Phe Ser Thr Val Val Phe Gly Leu Met
425 430 435

acg aag ccc ctc atc aat tta ctg ata ccc tca cca aag ctt aac aga 1757
Thr Lys Pro Leu Ile Asn Leu Leu Ile Pro Ser Pro Lys Leu Asn Arg
440 445 450

tcg gtc tct tca gaa ccg ctg act cca aac tcc atc aca atc cca ctt 1805
Ser Val Ser Ser Glu Pro Leu Thr Pro Asn Ser Ile Thr Ile Pro Leu

455 460 465 470

ctc ggg gaa agt cag gac tct gtg gcc gaa cta ttc agc atc aga ggt 1853
Leu Gly Glu Ser Gln Asp Ser Val Ala Glu Leu Phe Ser Ile Arg Gly
475 480 485

caa act tca caa ggt ggc gaa ccc gtt gct cga ccg agc agc cta cgc 1901
Gln Thr Ser Gln Gly Gly Glu Pro Val Ala Arg Pro Ser Ser Leu Arg
490 495 500

atg tta ctc aca aag ccc act cat acg gtg cac tat tat tgg aga aaa 1949
Met Leu Leu Thr Lys Pro Thr His Thr Val His Tyr Tyr Trp Arg Lys
505 510 515

ttc gac aat gct ttt atg cgt ccg gtc ttt ggt ggg cgt ggc ttt gta 1997
Phe Asp Asn Ala Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe Val
520 525 530

cca tat gtt ccc ggt tca ccg act gaa cga agc gtt cgc aac tgg gaa 2045
Pro Tyr Val Pro Gly Ser Pro Thr Glu Arg Ser Val Arg Asn Trp Glu
535 540 545 550

gaa gag acc aaa cag taaaaagatt ttcttgtgtg aatgatggtg aagagattag 2100
Glu Glu Thr Lys Gln
555

attctttgga tattcgtttt tcttatttct aatgtgtcac ctgggaagtt gttgaatgaa 2160

attatattat cgtctggttt tcgactttgc gcttgtggaa ggaatatttc ttctggattt 2220

tgcatggaaa cctcaatgat agggggtgtg atatttttgt tagaaactga gtcgtttgat 2280

gtatattgtt ggtaatgcag ctgggttttg ttttgtatgt atagtcatca agtgtgtatt 2340

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<210> 19
<211> 555
<212> PRT
<213> Torenia hybrida

<220>
<221> peptide
<222> (1)..(555)
<223> Amino acid sequence of protein regulating the pH of vacuoles

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Leu Trp Ser Ser Gly His Gly Ser Val Val Ala Ile Thr Leu Phe Val
20 25 30

Thr Leu Leu Cys Thr Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn
35 40 45

Arg Trp Met Asn Glu Ser Ile Ile Ala Leu Ile Ile Gly Leu Ala Thr
50 55 60

Gly Val Ile Ile Leu Leu Ile Ser Gly Gly Lys Ser Ser His Leu Leu
65 70 75 80

Val Phe Ser Glu Asp Leu Phe Phe Ile Tyr Ala Leu Pro Pro Ile Ile
85 90 95

Phe Asn Ala Gly Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe
100 105 110

Ala Thr Ile Met Met Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile
115 120 125

Ile Ile Ser Leu Gly Thr Ile Ala Phe Phe Pro Lys Met Asn Met Arg
130 135 140

Leu Gly Val Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr
145 150 155 160

Asp Ser Val Cys Thr Leu Gln Val Leu Ser Gln Asp Glu Thr Pro Leu
165 170 175

Leu Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser
180 185 190

Val Val Leu Phe Asn Ala Val Gln Asn Phe Asp Leu Pro His Met Ser
195 200 205

Thr Ala Lys Ala Phe Glu Leu Val Gly Asn Phe Phe Tyr Leu Phe Ala
210 215 220

Thr Ser Thr Val Leu Gly Val Leu Thr Gly Leu Leu Ser Ala Tyr Ile
225 230 235 240

Ile Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala
245 250 255

Ile Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe
260 265 270

Asp Leu Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser
275 280 285

His Tyr Thr Trp His Asn Val Thr Glu Asn Ser Arg Val Thr Thr Lys
290 295 300

His Thr Phe Ala Thr Leu Ser Phe Val Ala Glu Ile Phe Ile Phe Leu
305 310 315 320

Tyr Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Arg Phe Val Ser
325 330 335

Gly Ser Met Thr Thr Ser Ala Ala Val Ser Ala Thr Leu Leu Gly Leu
340 345 350

Val Leu Leu Ser Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser
355 360 365

Asn Leu Ala Lys Lys Ser Pro Leu Glu Lys Ile Ser Leu Arg Gln Gln
370 375 380

Ile Ile Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala
385 390 395 400

Leu Ala Tyr Lys Gln Phe Thr Arg Glu Gly Leu Thr Val Glu Arg Glu
405 410 415

Asn Ala Ile Phe Ile Thr Ser Thr Ile Thr Ile Val Leu Phe Ser Thr
420 425 430

Val Val Phe Gly Leu Met Thr Lys Pro Leu Ile Asn Leu Leu Ile Pro
435 440 445

Ser Pro Lys Leu Asn Arg Ser Val Ser Ser Glu Pro Leu Thr Pro Asn
450 455 460

Ser Ile Thr Ile Pro Leu Leu Gly Glu Ser Gln Asp Ser Val Ala Glu
465 470 475 480

Leu Phe Ser Ile Arg Gly Gln Thr Ser Gln Gly Gly Glu Pro Val Ala
485 490 495

Arg Pro Ser Ser Leu Arg Met Leu Leu Thr Lys Pro Thr His Thr Val
500 505 510

His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala Phe Met Arg Pro Val Phe
515 520 525

Gly Gly Arg Gly Phe Val Pro Tyr Val Pro Gly Ser Pro Thr Glu Arg
530 535 540

Ser Val Arg Asn Trp Glu Glu Glu Thr Lys Gln
545 550 555

<210> 20
<211> 6298
<212> DNA
<213> Ipomea nil

<220>
<221> misc_feature
<222> (1)..(6298)
<223> Nucleotide sequence of promoter region of gene encoding for protein regulating the pH of vacuoles

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gaaaggtaaa gacaattgga tcatataaag gtgatgagta ttatgggaaa ccatcaaaag 120

ttgggcaaat tcccgtccca tttaagaaat tcctcgaatc taaaggcatt tgtgcataat 180

acacaatgtc aggcacacca caacaaaatg gtgtggaaga aaggtgaaat cgtactctaa 240

gggaaatggt taggagttag gtaaataatt gtacattgct tgtttcattg tggatatatg 300

cattaaaaac aacagcatac ttactcaata gggttcctag taaggttgtt ttttaaaaca 360

ccttatgaac tgtggacaag aaggaaacct agtttgagac atcttcacat tcagggttgt 420

caagctggag tgatgatata taatccacat gaaaataaat tatggatatc cagaaccatt 480

aatggttatt tcattggata tccagaaagg tctaaaggta catgtttatt gtcctaatca 540

taagtacgag gattgttgag tctggtaatg ctcgcttcat ttaaaatggc gaagtcagtg 600

ggagtgtggg agctcgtaat gttaaaatta aggagtcatt gatggttcta gattcatcaa 660

gtgatccttt tttcctgttg ttgttcctat tgttgcagtg cagtgtagcc ttatggaaat 720

actttggaac aacagcaact agatgctcaa attccacatg aggaagctat tgtaaatgaa 780

gatgaggttg aaactcaaga tgatgatcaa gtgaaatctc agcaggaagt gacattaagg 840

aggtctacta tagatagaaa agatcaacca ttcttgatga ctatattgtt tatacacttg 900

agcattgata attatccagt ctcatttaac caagccatac aggataataa ttctccttga 960

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ttacctttct gaatggtaaa ttaaaagagg aagtatacat ggattagccg taaggcttca 1080

tggccacagg aaaggaaaat ctggtatgta gattgaagat gtcgatctat ggattaaaac 1140

atgcttctag acaatggtac ttgagattgt catttggttt tgtagagatc actgttgatc 1200

ggtgtatcca cataaaggtt taatggaagc aagtttgtaa tcctagtatt acatgttaac 1260

gacattcttc ttgctgctaa taataaaagg gatgttgcgt gatgttaagg aatagctttc 1320

taagaacttt gaaatgaagg atatgggtga gacttcatat gtgattggaa taagaaatat 1380

tccgtaatag atcacatggg attttaggtt catcctagaa gactcacatt aacaaagttt 1440

tagaaagata caaactggaa atctgcaaag agggtcctgt gatacttaca aggcactaag 1500

gaccacatgc tcacctataa aatgaacgaa taacctagag gttataggtt attcggattc 1560

agactatgcc ggatgtttgg atacccgaaa atccacattc gaatatattt ttccacttgc 1620

tcgtggagca atatcttgga tgagtgtgaa ggagcctgtc attgctactt ccactataag 1680

ggcagaattt gtagcatgct ttgaggctag tagacactat aaaaattggc tgccttgtgc 1740

caacatcatt tgcatccagc tataagcatc tccatttcg aacatcattc gattcttata 1800

gctggatgaa gatgattcac tgcactttgg ttgcatattg aagctgcgat tgctatcgaa 1860

aacaaataac ctatctatat aaaacaaacg acttagattt agggaataag aaggaagata 1920

cttttttaa aatcccaaaa ttacctttta ggtttgacct gcaaataaca ctttaagatc 1980

aaatcagata aaatgtcata atcaatgatc aaattgaata attttagtag tcgaggatca 2040

aattggtaaa atccccatag tcgagggact aaaccagtaa ttttctcgcg tttgaacgtt 2100

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gtttttgttt tttgtttttt tatttcctta tccctccacc tcattttgaa gttaattatt 2340

attattaatt cattaatttt taaaatagag agactgcatt aacacaaaat tagccaatta 2400

ggtagcagaa ttaaatttaa acaaacaagt tggtttaatg taatttttgt caatttaatt 2460

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tggttaaata taacttataa gtgcattgtg tccaaatgat cacaagagtt aggccaactt 2640

ctttttcat ataggtgatt ctttttcga gtattacgta cacttcagtc ttgtcaacta 2700

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gcttgcgggt tagaaaatac acagcccaag cccgtccatg cgggctcgcg ggccttattt 3180

caaaaaaaaa aaaaatacta cgtattattc tattatttta tattcaaata gtctaatata 3240

aataaataaa aaaatcgtgt ttgaaaatta cttttttttt tatatatatt ttttaaaatt 3300

ttaatgttat atacgaagtg tgtgtaatat atatatatat atatatatat atatatatat 3360

atatatatat atatatatat attatttata ttatttatat ttatgtttat atttaaatac 3420

gggcatggct cgtcggctgg tccgttaggt ccgctctttt gtaggccatt tttttgtgtg 3480

accctaaatc gtctcaccgc gggacaagta tagggcagct tgcggacttc ggtccatttt 3540

gacatatata tatatatata tatatatata tatatatata tatatatata tatatatata 3600

tatatatata taacattaaa atttaaaaaa tatagatttt ttttaaacat gaaaaaaatt 3660

aatattattt atattaaatt atttgaatat aaaataaata atactttttt atagcttgcg 3720
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